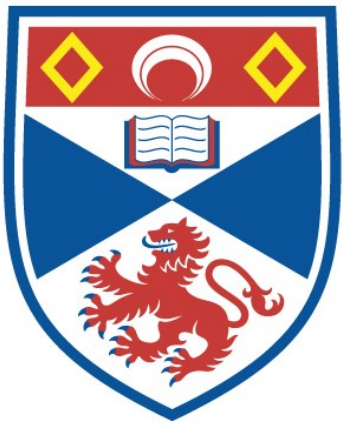
LIPID COMPOSITION OF FARMED AND WILD SALMON

Graham John Donachie

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1979

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to the

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in application for

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Abstract

The lipid composition in terms of component lipids and component acids - of farmed and wild Atlantic salmon (Salmo salar) was studied at various stages of their life cycle, up to and including sexual maturation. Lipids were extracted separately from flesh, liver and gonad organs for this study. The majority of samples examined in this work were from farmed salmon and to determine the influence of the diet on their lipid composition, a sample of the diet fed to farmed salmon was extracted and its lipids analysed.

The dietary lipid is composed mainly of neutral lipids, particularly triacylglycerols, with minor amounts of polar lipids (phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol and cardiolipin).

The fatty acid composition of the dietary lipid components suggested that the dietary lipid was a mixture of vegetable oils, seed oils and fish meal(s).

The major neutral lipids of salmon tissues were triacylglycerols, cholesterol and cholesterol esters while the dominant polar lipids were phosphatidyl choline and phosphatidyl ethanolamine, with smaller amounts of cardiolipin, phosphatidyl inositol, phosphatidyl serine and sphingomyelin.

The major fatty acids present in all samples examined included saturated acids (mainly 16:0 accompanied by 14:0 and 18:0), monoene acids (16:1, 18:1 and 20:1, with lower levels of 22:1 and 24:1), the n-6 group of acids, of which only 18:2 and 20:4 are consistently noticeable, and, at a much higher level, the n-3 group of acids with 22:6 > 20:5 > 22:5 >> other members.

Neutral lipids (mainly TG) were highest in the flesh and least in the liver where correspondingly polar lipids (PC and PE) were highest.

Variations occurred in fatty acid composition in relation to lipid class and lipid site (flesh, liver and gonads). Phosphatidyl cholines had the highest proportion of saturated acids at all three sites, whereas triacylglycerols had the highest monoene and n-6 polyene acid content. The phospholipids had very high levels of n-3 polyene acids at all three sites, whereas triacylglycerols had lower levels of these acids, although the difference was less marked in the gonads.

The total lipid levels and the lipid class composition of farmed and wild salmon, all of which had spent twelve months in sea water, differed only in the gonads, where the amount of lipid in the wild salmon was greatly increased. This was reflected in a higher proportion of triacylglycerols present in the wild salmon.

The major difference between the fatty acid composition of farmed and wild salmon was the higher concentration of linoleic acid in the farmed salmon triacylglycerols from all three sites compared to wild salmon triacylglycerols. This was attributed to the higher linoleic acid content in the diet of farmed salmon compared to that present in the natural marine diet.

With maturation there was a decrease in the total lipid content of flesh, little change in the liver, and an increase for the gonads. The proportion of neutral lipids decreased upon maturation, at all three sites.

During the period up to and including maturation, there appeared to be selective mobilisation of the fatty acids of hepatic polar lipids, C_{16} and C_{18} saturated and monoenoic acids increasing while the C_{20} and C_{22} n-3 polyenoic acids (particularly 22:6) decreased. In the gonadal phosphatidyl cholines, maturation was accompanied by an increase in the C_{20} and C_{22} n-3 polyenoic acids and a decrease in the saturated and monoenoic C_{16} and C_{18} acids.

No significant individual differences were observed in either total lipid levels or lipid composition in the flesh and liver of a group of male and female farmed salmon which originated from the same sea water pen, and had been fed the same diet.

To

Gill and Sarah

Declaration

I hereby declare that this thesis is a record of the results of my own experiments, that it is my own composition, and that it has not previously been presented in application for a higher degree.

Certificate

I hereby certify that Graham John Donachie has completed twelve terms of research work under my supervision, has fulfilled the conditions of the Resolution of the University Court 1967, No. 1 (St. Andrews) and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Research Supervisor

Career

I graduated in 1975 from the University of St. Andrews with the degree of Bachelor of Science.

I then studied for an M.Sc. degree in Clinical Biochemistry at the University of Newcastle-upon-Tyne which I completed in September 1976.

I was admitted as a research student in the United College, University of St. Andrews in October 1976, with a CASE studentship provided by the Science Research Council. Unilever Research, Aberdeen was the co-operating body.

The majority of this work was carried out in the Department of Chemistry, University of St. Andrews, under the supervision of Professor F.D. Gunstone, D.Sc., F.R.I.C.. In accordance with the conditions of the Science Research Council CASE award, I spent several periods at Unilever Research, Aberdeen under the supervision of Dr. M.C. Keith.

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Finally, I must thank the Science Research Council for my maintenance grant, Unilever Research for their assistance, both technical and financial, and to the Chemistry Department (St. Andrews) whose financial support enabled me to complete the final year of my research.

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Abbreviations

Fatty acids are reported in shorthand by showing the number of carbon atoms followed by a colon and a figure denoting the number of multiple bonds. The nature of unsaturation (shown by the letter c indicating cis olefinic) and its position relative to the carboxyl group are given in parenthesis.

eg. 18:1 (9c) is oleic acid

18:2 (9c, 12c) is linoleic acid.

In this thesis all the fatty acids discussed can be assumed to have the cis configuration unless otherwise stated.

Δ gives the position of the double bond counting from the carboxyl end and n-x shows its location from the methyl end where x is the position of the first double bond counting from the methyl end.

eg. 18:3 (n-3) is 18:3 (Δ9, Δ12, Δ15)

Ag^+TLC	Argentation thin layer chromatography
DEGS	Diethylene glycol succinate
EGSS	Ethylene glycol succinate
GLC	Gas liquid chromatography
GC-MS	Gas chromatography - mass spectrometry
M	Molecular ion
ECL	Equivalent chain length
TLC	Thin layer chromatography
R_f	retention index (TLC)
TG	Triacylglycerol
CE	Cholesterol esters
CHOL	Cholesterol
DG	Diacylglycerol

(cont.)

FFA	Free fatty acids
PC	Phosphatidyl choline
PE	Phosphatidyl ethanolamine
PS	Phosphatidyl serine
PI	Phosphatidyl inositol
SM	Sphingomyelin
CL	Cardiolipin
P	Phosphorus

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I. INTRODUCTION

1. Component Acids of Lipids from Aquatic Animals

The fatty acids derived from lipids of aquatic origin are more complex than those obtained from plants and land animals. The discovery in recent years of many fatty acids of novel structure from aquatic sources has served to emphasise this belief. These acids included: (a) methyl branched acids, (b) odd-chain length acids, (c) non-methylene interrupted dienoic acids, (d) C_{24}-C_{30} polyenoic acids (and other unusual polyenoic acids), (e) acids with trans unsaturation, and (f) acids containing a furanoid ring system[1a]. These acids are usually minor components of aquatic lipids, although their levels can increase significantly under special circumstances.

The Major Acids

(i) Structure

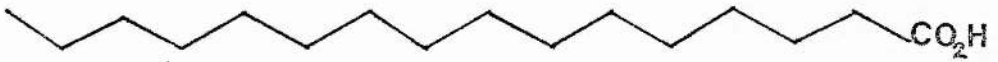
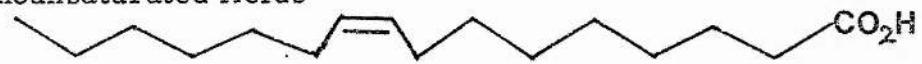
It has also been reported[1b] that fats of aquatic origin are characterised by the following features:

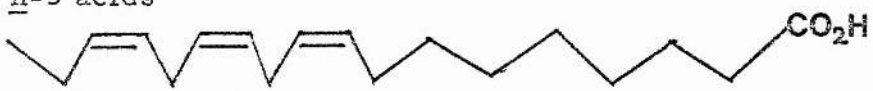
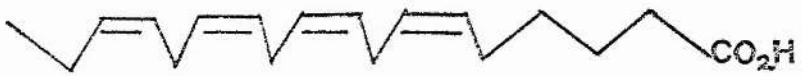
(a) The component acids, usually exceeding 20 in number, vary in chain length from C_{14} to C_{24} and may also contain minor amounts of acids with 12, 26 or an odd number of carbon atoms;

(b) Saturated acids (~25%) are mainly palmitic (16:0, 15-20%) accompanied usually by myristic (14:0) and stearic (18:0);

(c) Monoene acids (35-60%), whilst predominantly C_{18} (mainly Δ9 and Δ11), also include C_{16} (Δ9), C_{20} (Δ9 and Δ11) and C_{22} (Δ11 and Δ 13) acids;

Table 1. Names and Structures of Some Fatty Acids Commonly Occurring in Lipids of Aquatic Origin

Structure	Name	Symbol
Saturated Acids		
	Tetradecanoic acid (Myristic acid)	14:0
	Hexadecanoic acid (Palmitic acid)	16:0
	Octadecanoic acid (Stearic acid)	18:0
Monounsaturated Acids		
	Hexadec-9-enoic acid (Palmitoleic acid)	16:1 (n-7)
	Octadec-9-enoic acid (Oleic acid)	18:1 (n-9)
	Eicos-11-enoic acid (Gadoleic acid)	20:1 (n-9)
	Docos-11-enoic acid (Cetoleic acid)	22:1 (n-11)

Structure	Name	Symbol
n-6 acids		
	Octadeca-9, 12-dienoic acid (Linoleic acid)	18:2 (n-6)
	Octadeca-6, 9, 12-trienoic acid (γ-Linolenic acid)	18:3 (n-6)
	Eicosa-5, 8, 11, 14-tetraenoic acid (Arachidonic acid)	20:4 (n-6)
	Docosa-4, 7, 10, 13, 16-pentaenoic acid	22:5 (n-6)
n-3 acids		
	Octadeca-9, 12, 15-trienoic acid (α-Linolenic acid)	18:3 (n-3)
	Octadeca-6, 9, 12, 15-trienoic acid	18:4 (n-3)
	Eicosa-5, 8, 11, 14, 17-pentaenoic acid	20:5 (n-3)
	Docosa-7, 10, 13, 16, 19-pentaenoic acid	22:5 (n-3)
	Docosa-4, 7, 10, 13, 16, 19-hexaenoic acid	22:6 (n-3)

(d) The remaining acids are mainly polyenes of the n-6 (18:2 to 22:5) and n-3 (18:3 to 22:6) families. The latter group predominate and most lipids from aquatic sources contain the 20:5 and 22:6 n-3 acids as major components.

The nature and the relative proportions of acids from aquatic animals are very variable and they are influenced by many external factors, hence the above statements tend to oversimplify a complex situation.

The origin of these fatty acids and the external factors which regulate their relative proportions and composition, are now considered.

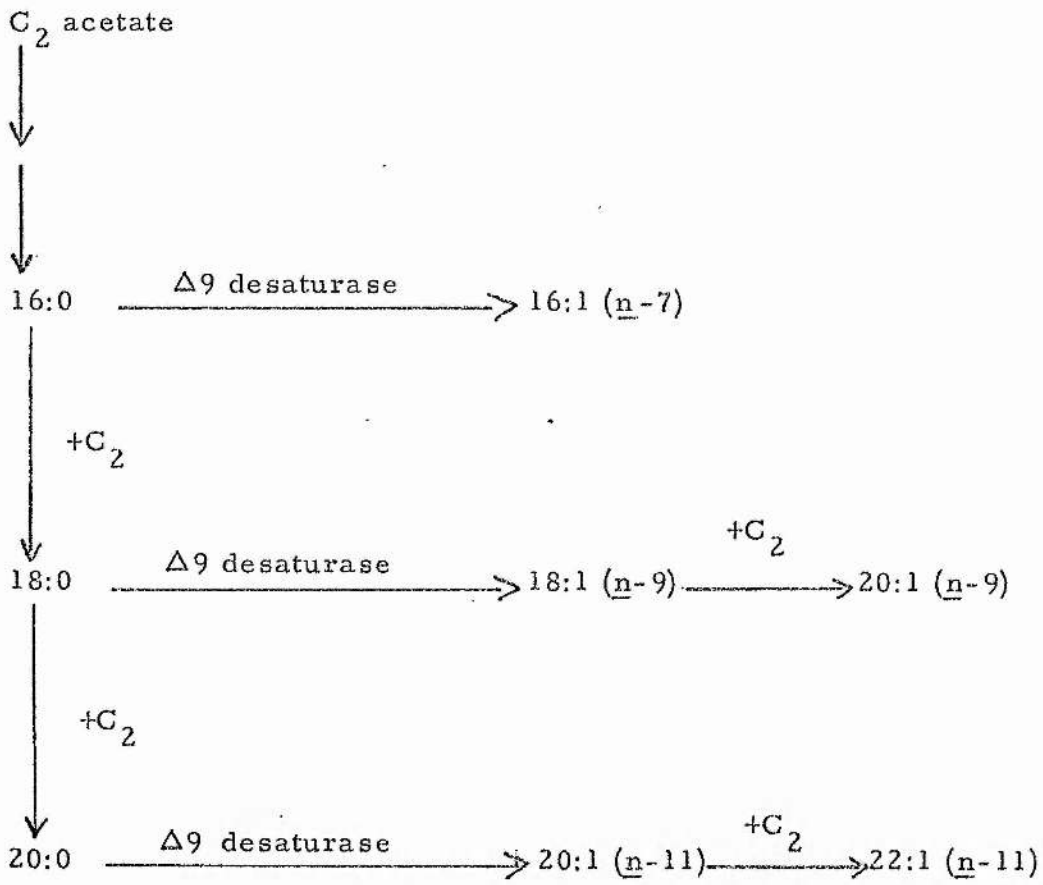
(ii) Origin

The fatty acids from the lipids of aquatic animals are either synthesised directly from C_2 precursors (endogenous) or they are derived from the diet (exogenous).

(a) Endogenous acids

Marine organisms, in common with all life forms so far examined, have the capacity to synthesise fatty acids de novo from C_2 units such as acetate, derived from dietary protein or carbohydrate. The end product of the fatty acid synthetase is usually palmitic acid (16:0). Fatty acids of chain length longer than C_{16} are then synthesised by further stepwise addition of two carbon units.

Saturated acids are readily desaturated - mainly by a $\Delta 9$-desaturase present in liver microsomes.

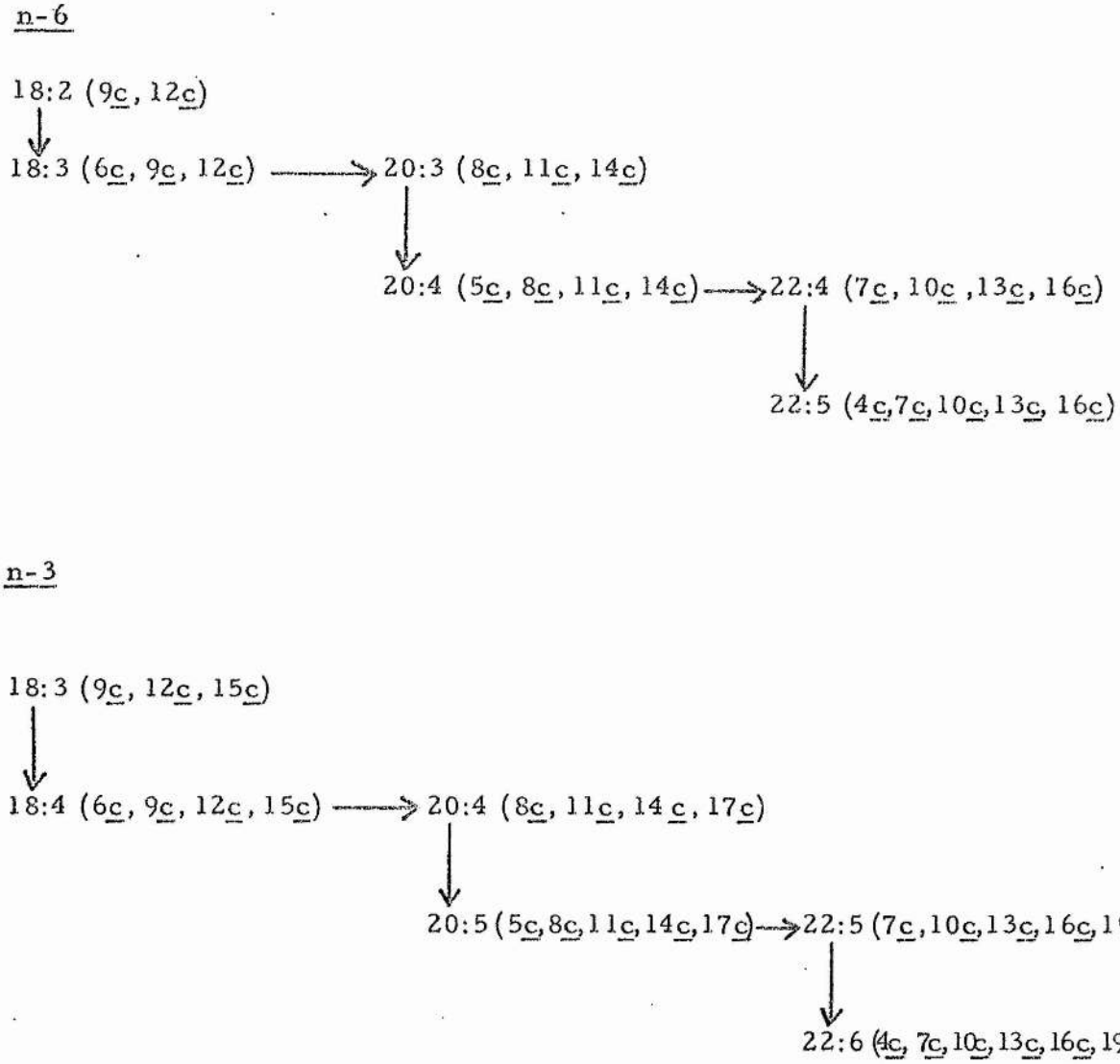


In the 22:1 acids found in aquatic sources the Δ11 (n-11) C_{22} acid is more common than its Δ13 (n-9) isomer. These acids are likely to result from Δ9 desaturation and chain elongation of the C_{20} and C_{18} saturated acids respectively. Cowey and Sargent[2] suggested that the Δ11 (n-11) C_{22} acid, formed by this mechanism, possibly originated in the zooplankton (the major food of most fish).

(b) Exogenous acids

Fish cannot accomplish the de novo synthesis of polyunsaturated fatty acids of either the n-6 or n-3 series, as they are incapable (like other animals) of introducing a double bond between the Δ9 and methyl end of the fatty acid molecule. However, with a few

exceptions, they can further elongate and desaturate dietary 18:2 (n-6) or 18:3 (n-3) as outlined by the pathways shown below.



Polyunsaturated fatty acids of the n-6 and n-3 families are essential dietary factors for fish. In the trout[3], deficiency of these result in a series of pathologies including erosion of the caudal fin, myocarditis and shock syndrome. Accompanying these

physical changes is a high level of 20:3 (n-9) acid in the tissues, characteristic of essential fatty acid deficiency. This n-9 acid is produced from oleic acid by the elongating and desaturating systems which normally operate on the C_{18} diene and triene acids.

The essential fatty acid requirements of aquatic animals vary with the species. In the rainbow trout Salmo gairdnerii[4,5] and the prawn Penaeus japonicus[6], linolenic acid (18:3 n-3) is more effective than linoleic acid (18:2 n-6) in curing symptoms caused by essential fatty acid deficiency, whereas both 18:2 (n-6) and 18:3 (n-3) are equally effective for the carp Cyprinus carpio[7]. In contrast to these animals, neither 18:2 (n-6) nor 18:3 (n-3) exert a growth promoting effect for the red sea bream, Chrysophys major[8,9].

The varying effect of dietary 18:3 (n-3) on the growth of different aquatic animals results from their differing ability to elongate and desaturate dietary 18:3 (n-3) to 22:6 (n-3). Recently, Kanazawa et al[10] carried out a series of experiments on several species of marine and fresh water fish, assessing their ability to carry out this bioconversion. Linolenic acid was converted to 20:5 (n-3) and 22:6 (n-3) intensively in the rainbow trout (Salmo gairdnerii), moderately in the ayu (Plecoglossus activelis), eel (Anguilla japonica) and prawn (Penaeus japonicus), but only slightly in the red sea bream (Chrysophys major), rockfish (Sebasticus marmoratus) and globefish (Fugu rubripes rubripes). The marine flatfish, plaice[11] and turbot[12] are also reported to be incapable of carrying out this bioconversion. Remarkably, this ability to

convert C_{18} fatty acids to polyunsaturated ones, in this case linoleic to arachidonic acid, is also lacking in the extreme carnivores cat[13] Felis cattus and lion[14], Panthera leo, perhaps an example of biochemical evolution along parallel lines between divergent forms, even although they are in quite separate environments. However, diets rich in highly unsaturated acids 20:5 (n-3) and 22:6 (n-3) are more effective than 18:2 (n-6) or 18:3 (n-3) in promoting weight gain in fish such as prawn[15] and red sea bream[9]. In rainbow trout, 20:5 and 22:6 (n-3) have almost equal[16], or superior[17] effects on weight gain to 18:3 (n-3). This higher essential fatty acid activity of 20:5 (n-3) and 22:6 (n-3) compared to 18:3 (n-3) in fish, forms a direct analogy with omnivorous mammals where arachidonic (20:4 n-6) has greater essential fatty acid activity than its precursor 18:2 (n-6).

As marine fish generally receive a sufficiency of long chain polyunsaturated fatty acids in their diet, the ability to convert, for example, 18:3 to 22:6 (n-3) is perhaps unnecessary and fish such as red sea bream and globefish may therefore never have lost or never acquired the ability to carry out this bioconversion, as it is of little immediate value to them.

(iii) Influence of the Environment

Aquatic animals are exposed to fluctuating environmental influences such as temperature, diet, pressure (depth), and oxygen concentration, all of which have a profound effect upon their component lipid and fatty acid composition.

(a) Diet

The relationship between the fatty acid composition of lipids and the conditions under which the organism exists, has led to the concept of marine and freshwater types of lipids[18,19]. According to this, there is a higher proportion of the C_{16} and C_{18} acids in the lipids of freshwater organisms (fish and the planktonic crustaceans which provide them with food) by comparison with marine organisms. According to Ackman[20], the clearest characteristic differences between the lipids of marine and freshwater fish are: (1) the higher proportion of the essential linoleic (18:2 n-6) and linolenic (18:3 n-3) acids in the lipids of freshwater fish and (2) the predominance of n-6 polyene acids (18:2, 20:4, 22:4 and 22:5) in freshwater fish and of n-3 polyene acids (18:3, 20:5, 22:5 and 22:6) in marine fish. As marine and freshwater fish probably do not differ in their mechanisms for deposition, synthesis and interconversion of fatty acids, the differences in fatty acid composition are probably of dietary origin[21].

Migratory fish in the course of their lives change several times between a freshwater and marine environment. Young coho salmon [Oncorhynchus nerka] inhabiting freshwater contain a high percentage of linoleic acid and other n-6 polyene acids of this family in their lipids - typical "freshwater" fish. The mature coho caught in the sea is similar to marine fish, having a high content of "marine" fatty acids, 20:5 and 22:6 (n-3).

Anguilla anguilla exist in freshwater and marine forms, with the fatty acid composition expected of their environment. Under controlled conditions however, it has been shown that their fatty acid composition depends on their diet and not on the salinity of the water in which they are kept[22]. Thus diet is the overriding factor and in general fish lipids are usually similar to the lipids of the food. However, certain fish[22] can modify the ingested lipid considerably. This is best illustrated in Salmo salar[23] where the body lipid of the "parr" form (early freshwater) is like the lipid that they ingest, but the "smolt" (later freshwater) lipid is more like that of the adult (marine) form, though there has been no change of diet or environment

(b) Wild vs Farmed

Differences between the artificial diets of farmed fish and the natural foods of wild fish may directly influence the composition and physical condition of the fish which consume them[24,25].

Reared fish often have higher fat (lipid) contents than their wild counterparts[26]. This has been reported for salmonid species[25], herring[28] and brooktrout[27], where the body lipid content of the reared fish was higher than that to be expected from the difference in lipid contents of the two types of food. Total fat was also found to increase with increasing size for reared trout[27] and herring[28], but not in the same species taken from the sea. The increased fat was mainly neutral lipid (triacylglycerol), the major storage form of excess calorific input.

Phillips[29] believed that the higher lipid content of reared trout compared to those from the wild, implied that the reared ones consumed more calories, were less active, or both. One would expected reared fish to expend considerably less energy than wild ones avoiding predators and searching for food, although behavioural studies suggested that reared herring were the more active[28]. However, the higher lipid content of the diet, and its easier availability for the reared fish, probably means that despite its greater activity, the reared fish still has sufficient energy to store in the form of triacylglycerol (TG). Although the total lipid level increased with increasing size for reared herring, the total TG/total lipid decreased, implying that as the growth rate increased the excess energy available from the food and not needed for promoting growth, declined.

Other differences between wild and farmed fish have been reported. The fatty acid compositions of various organs of wild and cultivated "ayu" sweet smelt (Plecoglossus altivelis) differ markedly in the TG fractions, although this was not observed for the phospholipids[30]. In muscle, the total C_{14} and C_{16} acids exceeded 50% of the total fatty acids for wild fish, but was around 40% for cultivated fish. In the fats of liver, abdomen and sexual organs, the wild had higher amounts of 14:0, 16:1, and 18:4 while the reared showed more 16:0, 18:1 and 18:2.

The differences between wild and cultivated fish become more extreme as the period of artificial rearing is increased, but some at least, soon vanish if the cultivated fish are transferred

to a natural environment[31]. However, introduction of young coho salmon (Oncorhynchus keta) fry into seawater was reported[32] to cause a substantial diminution in the levels of polyunsaturated fatty acids, a phenomenon not observed for wild fry in the marine environment. The authors suggested that the stresses imposed by confinement, general handling procedures and partial starvation were responsible.

(c) Temperature

It is now well established that an inverse relationship exists between the temperature of a tissue and its fatty acid composition. In general, as the temperature of the medium falls, there is an increase in the proportion of the highly unsaturated long chain C_{20} and C_{22} fatty acids compared with the less unsaturated C_{16} and C_{18} acids. This effect has been observed in goldfish muscle[33], brain[34,35] and intestinal lipids[36], and in rainbow trout[37] and several types of marine fish[38]. The increase in fatty acid unsaturation may be a means of adjusting membrane viscosity within the range necessary for metabolic processes[38]. Roots[35] suggested that this modification of the fatty acid composition assists in the maintenance of proper membrane fluidity and permeability for efficient functioning of the nervous system.

Roots[35] also emphasised that the levels of the various phospholipids in these membranes did not change with decrease in temperature, only the degree of unsaturation of their fatty acids. However, De Torrengo and Brenner[39] found an actual

decrease in the amount of polyunsaturated acids in the liver microsomes of fish kept at 15°C as compared to those kept at 30°C, a discrepancy also noted by other workers[33].

Poikilothermic animals such as fish do not have a constant body temperature and the adaptation of physiochemical properties of their membranes to ever changing temperature therefore has considerable survival value. The adaptation of the fish to alterations in the temperature of the medium could occur on the one hand, by inner mechanisms which control the degree of unsaturation of their fatty acids, perhaps by increased activity at lower temperatures[39,40] of those systems responsible for long chain polyunsaturated fatty acids compared to fatty acid synthetase, or by selective absorption of certain fatty acids by the intestine at lower temperatures[37]. On the other hand, the temperature affects the unsaturation of fatty acids of fish lipids throughout the food chain. Farkas and Herodek[41] demonstrated that C_{20} and C_{22} polyunsaturated fatty acids of certain freshwater crustaceans increased with decreasing water temperature. Kayama et al[42] feeding brine shrimp to guppies (Lebistes reticulatus) maintained at different water temperatures, found that fish maintained in warmer water showed a relative decrease in 16:1, 18:1 and 22:6 fatty acids.

(d) Pressure (Depth)

Lipid composition also changes with pressure (depth). Lewis[43] showed that saturated fatty acids of medium chain length

($C_{16:0}$ and $C_{18:0}$) and also long chain polyunsaturated acids (C_{20} and C_{22}), decrease with species that live in increasingly deep water, while oleic acid ($C_{18:1}$) increases. These findings were supported by other workers[44] who examined the polyenoic fatty acid contents of liver neutral lipids in six species of fish. The presence of increased levels of oleic acid at increasing depths is thought to indicate the presence of large amounts of wax esters[45], known to be rich in this acid.

However, the precise relationship between the fatty acid composition of a marine organism and its pressure (depth) is still not precisely determined. Various other factors such as temperature (highly variable at different depths of the ocean), the nature and amount of the food supply, adaptation to darkness as well as high hydrostatic pressure[46], tend to cloud this relationship. Indeed, little is still known about the membrane lipid composition as a function of depth in marine water[47], nor the effect of hydrostatic pressure on biomembrane structure.

(e) Sex and Season

It is very difficult to attempt a systematic appraisal of "seasonal variation" since the effects embrace not only all of the environmental influences, but also those of maturity and spawning.

The qualitative and quantitative nature of the lipids in the natural diet may vary throughout the year and this can be reflected in the fatty acid composition of the body lipids of the fish. Lovern[48] found a rapid rise in both the fat content and

its degree of unsaturation, prior to the spawning season, as a result of the ingestion of large amounts of unsaturated lipid during this period. The content of polyunsaturated fatty acids in the hepatopancreas (foodstore) of the yellow clam[49] (Mesodesma mactroides) has been reported to increase in summer, co-inciding with an increase of phytoplankton in the sea water. Slight variations have been observed[50] in the proportions of 20:1 fatty acids in the flesh lipids of cod caught during summer relative to the proportion of those caught at other times.

However, for anadromous fish like the salmon, the stage in its life cycle must be considered as well as the season. The muscle lipid content of wild Onch Masu parr of both the residual and seaward migration type, during freshwater habitation, decreases gradually from summer to winter as the diet decreases[51]. However, in spring the seaward migratory type of Masu salmon parr has a lower lipid content in its muscle compared with the residual type of parr, and in particular, lower levels of palmitoleic and oleic acids. This is a reflection of the approaching smolt transformation where the consumption of lipid reserves (mainly triacylglycerols) increases during the seaward migration and among the fatty acids, the monoenoic ones are chiefly consumed. Other workers have also reported[52] the preferential utilisation of long chain monoenoic acids by migrating salmon.

Changes have been observed in fatty acid composition in relation to both sex and season. Outside the breeding season it was found that the liver oils in female cod contained increasing amounts of C_{20} and C_{22} monounsaturated fatty acids as the lipid content of the liver increased. In males this seasonal trend was not exhibited, and no other fatty acids varied in this way. The fatty acids of flesh lipids varied independently of sex[50]. De Witt[53] observed an increase in mono and polyunsaturated fatty acids in the hepatic lipids of cod during the winter and summer months, an abrupt decrease taking place during the March spawning season. No significant variation in saturated fatty acids was observed throughout the annual cycle.

The most marked differences between the sexes occur at times of the year when the gonads are active, and particularly when preparing for spawning. The females of many species are frequently larger than the males because the males tend to mature earlier and this tends to slow their growth rate. This has been observed for Salmo trutta[54] and both freshwater and saltwater fish in general[55]. Female gonads of the salmonid species generally grow to a larger size than those of the male and therefore they consume larger amounts of their lipid reserves, both for energy and growth, during spawning migration[56]. However, in spite of differences in gonad size, Lovern[23] actually found more body lipid used up by male Salmo salar during spawning migration, probably because of repeated fighting amongst themselves as they ascended the river.

During the maturation of fish, the lipid contents of both the liver and gonads increases, the latter decreasing after spawning[57]. In rainbow trout (Salmo gairdnerii), maturation of the female gonads is believed to take place at the expense of stored visceral lipid (mainly TG) which is transferred to the liver, where it is then extruded in the blood in the form of lipoprotein and transported to the ovary[58]. Lovern[59] believed that a high selectivity of transferred individual fatty acids was shown from the body lipid of Salmo salar to the developing ovaries, those fatty acids mobilised but not required, being used as fuel. As the ova of this species mature, the relative proportions of C_{18} unsaturated acids within the lipids show a steady increase, while C_{16} acids, both saturated and unsaturated, decline[23].

Presumably because of differing requirements of the gonads, some differences have been observed in the lipid fractions of the two sexes during maturation. In Salmo salar the concentration of palmitoleic acid in the lipid reserves drops steadily while C_{22} acids show a marked rise in the male and little change in the female[59]. In the cod (Gadus morhua), long chain monounsaturated fatty acids from the liver are preferentially utilised for the synthesis of particular lipids in the developing eggs[60].

Maturation always depletes the lipid reserves of the fish. This effect is particularly marked for salmonid fish which refuse to feed while ascending the rivers to spawn. This voluntary starvation causes such massive depletion of the lipid reserves of the fish that the usual result is a post-spawning death.

Post-spawning pink salmon (Oncorhychus gorbuscha) are reported[61] to lack the ability to synthesise triacylglycerols. This correlates with a decrease in liver triacylglycerols, used up by starving fish, their fatty acids being mobilised in the form of cholesterol esters as energy sources.

2. Marine Lipid Classes

(i) Neutral Lipids

a) Triacylglycerols

In triacylglycerols all three glycerol hydroxyl groups are esterified to fatty acids (see Fig. 1).

Triacylglycerols are found in all life forms, including marine organisms. However, unlike their terrestial counterparts, triacylglycerols are by no means the only or even the major reserve lipid in marine animals. Crustacean zooplankton and other crustaceans usually contain triacylglycerols in varying amounts, but wax esters are frequently the major neutral lipid class. Nevertheless, triacylglycerols are present in nearly all species of fish and they are major constituents of adipose tissue and marine oils.

Stereospecific analysis[62] has revealed that marine triacylglycerols are basically similar to those of terrestial mammals, in having saturated acids concentrated at positions 1 and 3 and polyunsaturated fatty acids concentrated on position 2. In general, marine triacylglycerols are characterised by relatively higher proportions of n-3 polyenoic acids, notably 20:5, 22:5 and 22:6, compared to terrestial plants and animals which contain higher proportions of C_{16} and C_{18} acids.

b) Cholesterol and Cholesterol esters

Glycerol is by far the major alcohol found esterified to long chain fatty acids in marine organisms, but another important alcohol which can be found in esterified and unesterified forms is cholesterol (see Figure 1).

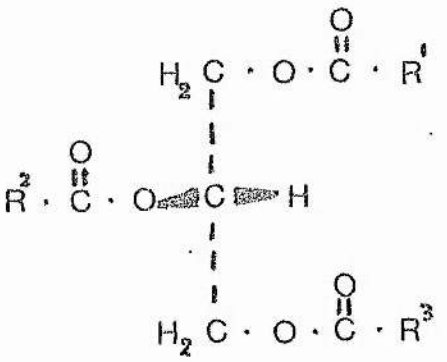
Cholesterol is important as a membrane constituent and as a precursor of the steroid hormones and bile acids. Cholesterol is present in the blood stream mainly as an ester, being involved in lipid transport as an integral constituent of lipoproteins. It has been reported[63] that the VLDL (very low density lipoprotein) of sardine are very rich in cholesterol esters in contrast to land mammals where the cholesterol esters are mainly associated with the LDL (low density lipoprotein) fraction. However, the LDL and all the other serum lipoproteins of Latimeria have a very low content of cholesterol ester[64]. The cholesterol esters of land mammals are very rich in polyunsaturated fatty acids and this is true also for the cholesterol esters of the sardine and Myxine, but not the coelocanth[64].

Cholesterol and its esters are usually present as low proportions of the total neutral lipid, but in special circumstances their levels may be greatly increased[1a].

c) Wax esters

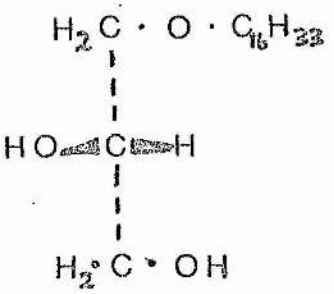
A long chain fatty acid esterified to a long chain fatty alcohol is termed a wax ester. Marine wax esters usually contain only primary fatty alcohols which are predominantly saturated and monounsaturated. The composition of the

Figure 1. Structure of neutral lipids



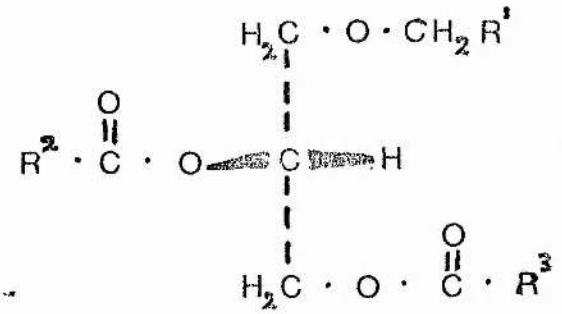
R^1, R^2, R^3 = fatty alkyl units (saturated and unsaturated)

1, 2, 3-triacyl-sn-glycerol

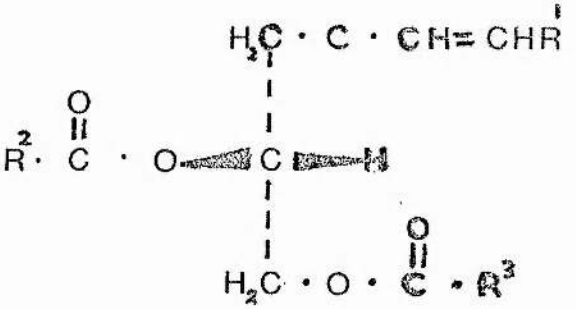


Chimyl alcohol

1-hexadecyl-sn-glycerol

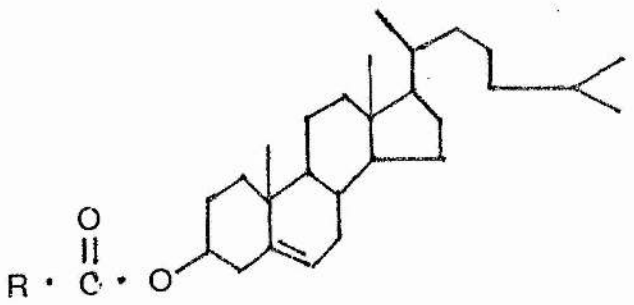


1-alkyl-2, 3-diacyl-sn-glycerol



1-alk-1'-enyl-2, 3-diacyl-sn-glycerol

Alkyl and alk-1'-enyl diacylglycerols



Cholesterol ester

fatty acid moiety is usually more complex, though not as complex as typical marine triacylglycerols. In deep sea crustaceans and mesopelagic and bathypelagic fish, 16:0 and 18:1 are the major alcohols, whereas the major acids are 16:1 and especially 18:1.

Zooplankton crustaceans such as the calanoid copepods, are particularly rich in wax esters. The presence of large amounts of wax esters in this species is believed to be the result of adaptation to two different types of environment[65]. Firstly, large amounts of wax esters are accumulated in copepods which inhabit an environment such as the Arctic, where food (plankton) is only plentiful for short periods of frequent feeding, followed by a famine. Secondly, in mid to deep oceanic waters (meso and bathypelagic regions) where both animal and plant life is scarce. Large amounts of wax esters are stored as lipid reserves in crustaceans which inhabit this environment (copepods, euphausids, mysids and decapods). For similar reasons mesopelagic and bathypelagic fish such as deep sea cod (moridae) and myctophids have also been reported to contain wax esters[45].

The calanoid copepods play an important role in the marine food chain as they are the major food of several species of fish including young salmon, herring and mackerel. It follows that wax esters are one of the dominant dietary constituents of these fish.

(d) Glyceryl ethers

The non-saponifiable fraction of many marine oils contain glyceryl ethers (alkylglycerols) derived from the corresponding alkyldiacylglycerols (see Fig. 1).

Chimyl, batyl and selachyl alcohols are glyceryl monoethers derived from the long chain alcohols corresponding to $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ respectively. These major naturally occurring glyceryl ethers are usually esterified with one or two fatty acids. Both alkyl and alk-1'-enyl glyceryl ethers (particularly their diester forms) are found in high proportions in the lipids of certain marine species. The fatty acids of alkyldiacylglycerols usually cover a wider structural range than the alkyl moieties.

Alkyldiacylglycerols (diacyl alkyl glyceryl ethers) are found in large amounts in the liver and musculature of the spur dogfish, Squalus acanthias and in the liver oil of the ratfish Chimaera monstrosa. High proportions of these lipids are found in a variety of(squaloid) sharks, especially those from meso and bathypelagic regions.

Wax esters are also found in marine species inhabiting these regions and a marked similarity between these two neutral lipids is in the alkyl moieties of alkyldiacylglycerols (16:0, 16:1 and 18:1) and the alcohol moieties of wax esters.

In marine organisms wax esters and alkyldiacylglycerols, as well as fulfilling the same basic role of neutral lipids in all life forms ie. as energy reserves, are believed to generate buoyancy and help aquatic animals achieve an advantageous,

weightless condition.

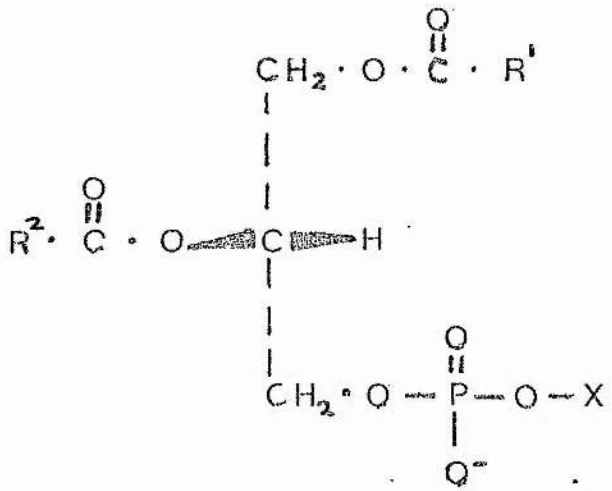
(ii) Polar lipids (phospholipids)

The most abundant phospholipids, found in every living cell, are mixed esters of the alcohol glycerol with fatty acids, phosphoric acid and an amino alcohol (nitrogenous base) or a polyhydroxyl compound (see Fig. 2). An important, but rather less abundant group, are amides of long chain bases such as sphingenine (Sphingophospholipids).

Various groups occur at position X, giving rise to a wide range of derivatives including phosphatidyl choline (X=choline), phosphatidyl ethanolamine (X=ethanolamine), phosphatidyl serine (X=serine), phosphatidyl inositol (X=inositol) and the major sphingophospholipid, sphingomyelin (X=choline). The fatty acid residues esterified at positions 1 and 2 of the glycerol molecule usually differ in their chain length (usually C_{14} to C_{22} for marine lipids), degree of unsaturation and branching. In marine organisms, polyunsaturated fatty acids are usually concentrated at position 2 while palmitic acid is concentrated on position 1. Phospholipids (plasmalogens) are also found in which the long chain alkyl group is linked to the glycerol backbone by a vinyl ether linkage at position 1.

In common with other life forms, the major marine phospholipids are phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol and sphingomyelin.

Figure 2. Structure of polar lipids



R^1, R^2 = fatty alkyl units (saturated and unsaturated)

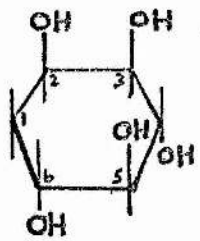
Glycerophospholipids (phosphatidyl-X)

X = —H Phosphatidic acid

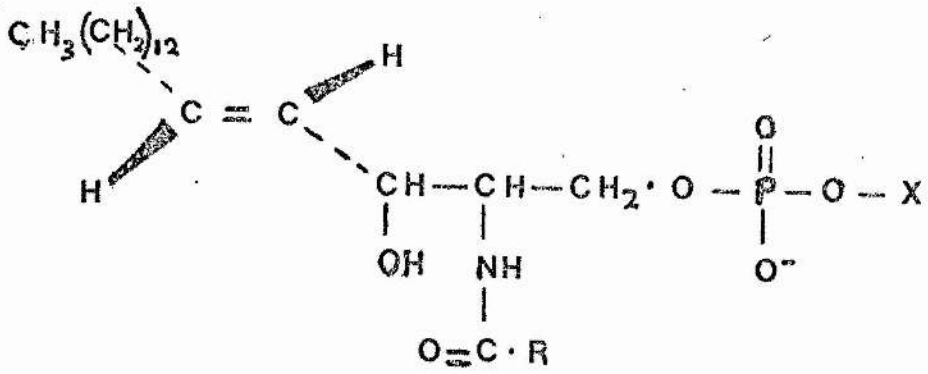
$-CH_2CH_2\overset{+}{N}(CH_3)_3$ Phosphatidyl choline

$-CH_2CH_2 \cdot NH_2$ Phosphatidyl ethanolamine

$-CH_2CH(NH_2)COOH$ Phosphatidyl serine



Phosphatidyl inositol



Sphingophospholipids

Phospholipids are an integral part of all cell biomembranes and as such are viewed as elements of "constancy". Unlike the triacylglycerols which are depleted during fasting, the phospholipids are not used as reserve supplies of energy, except under conditions of physiological emergency, for example, during the spawning migration of salmonid fish. The fundamental role of the phospholipids in cellular structure is such that depletion of these compounds may contribute to the ultimate death of this species.

Marine organisms have a preponderance of n-3 acids as compared to land animals and plants, where n-6 acids predominate. Marine phospholipids are particularly rich in members of the n-3 family of polyene acids and it has been speculated[65] that this preponderance of n-3 acids in marine life is intimately connected with the requirement of membranes to retain their fluidity at the relatively low and constant temperatures which are a feature of marine life. An n-3 acid will be more unsaturated than an n-6 acid for a given chain length and as the fluidity of a lipid is related to the degree of unsaturation of its fatty acids (as well as other factors), the high n-3 content may simply reflect an adaptation to the constraints of marine life.

Phospholipids are also believed to play a specialised function in gill tissue where they are intimately involved in the osmoregulatory activity of this organ.

II. RESULTS AND DISCUSSION

1. The life cycle of the salmon (wild)

Two distinct environments dominate the salmon's life history.

(1) A freshwater environment where the salmon's nursery and reproductive phases occur.

(2) The marine environment, where its main feeding phase and its greatest weight gain occurs.

Spawning or egg laying, usually takes place in late autumn or early winter. The eggs are laid in well protected nests or redds under several inches of gravel on the river bed. The fertilised eggs usually hatch in late March or early April and the alevins make their way up through the gravel to emerge as fry, four or five weeks later, when the yolk sac has been absorbed. During the first few weeks there is a high mortality due to starvation, predation and competition for space.

After one year of life, the fry, now known as parr, can be recognised by the dark blotches or "parr" marks along each side of the body. The salmon parr may remain at this stage for anything up to five years, depending upon stream conditions, before going to sea as smolts. During the smolt transformation, profound physiological changes take place in the young salmon's

body, preparing it for the journey downstream to the sea and the brackish and saltwater conditions it will meet on the way. The physical appearance also changes, the skin turns silvery (concealing the parr markings) and pectoral and caudal fins turn black.

Those fish returning to spawn after one year in the sea are termed "grilse", while those spending two or more years in the sea before returning to freshwater, are termed salmon.

The adult male and female salmon change in appearance after entering freshwater, and as spawning time approaches. Some of their bones grow substantially, including the main bones of the jaw. This is particularly noticeable in the male which develops a hooked lower jaw.

While ascending the rivers to spawn the salmon do not feed and concomitantly suffer massive depletion of their body reserves. This tremendous reduction in body weight (up to 40%) which the salmon suffer is believed to be the cause of the high mortality rate, particularly among males. Another theory for the death of post-spawning salmon ("kelts") is that the fish suffer from Cushing's disease caused by excessive secretion of ACTH, released by an overstimulated pituitary.

Salmo gairdnerii usually survive spawning because they seem able to reverse the process of vascular degeneration (occurring at spawning time) when they enter sea water, unlike most Pacific salmon which die. The difference may be that the latter are unable to excrete the excess (lethal) cortisol after spawning[66].

2. Salmon "farming"

"Farmed" salmon are hatched and reared in captivity and will never experience a river or the open sea. However, only an experienced eye can distinguish them in physical appearance and quality from their wild counterparts.

Atlantic salmon (Salmo salar) have been chosen for large scale commercial farming by a number of enterprises. Salmon are a "high quality" fish liable always to command a good price in the fish market, thus making it possible to recoup some of the massive expenditure which fish farming requires; the techniques for salmon husbandry (including disease problems) and their nutritional requirements have been well researched.

The basic requirements for a salmon farming site include clean freshwater, deep unpolluted tidal sea water and shelter.

The farming cycle begins in the hatchery (freshwater) ponds where brood fish are stripped of their eggs (early November). These are brought indoors immediately after fertilisation and placed in gently flowing water in shallow trays. The eggs are carefully distributed in a single layer in the flutes of finely corrugated gauze or wire mesh.

The natural conditions underneath the gravel of the stream bed are simulated as closely as possible, by keeping the incubating room in darkness throughout the ova and subsequent alevin stages. Assuming normal water temperatures, eggs fertilised in early November will have reached the eyed stage by early February and the alevin begin to hatch out of their yolk sacs by the end of March. The alevin slip through the wire mesh into the tray below, where they continue to use up the food reserves in their yolk sacs for the next four or five weeks. The active young fish, nearly at the fry stage of life, are then transferred to glass fibre tanks (one metre square) adjacent to the ova trays and exposed to the light for the first time.

The fry remain in these indoor tanks, supplied with a constant water flow, for 7-8 weeks. They are fed regularly during daytime with a specially formulated diet. By early summer, the young fish reveal the characteristic parr markings (blue bars and red spots on their backs) and are ready to be moved outside and distributed around a battery of larger glass fibre tanks. The parr grow rapidly throughout the summer and following winter on the regular supply of feed, which is gradually increased in size.

Some fish smolt in the following spring, others continue feeding for another summer and winter and become second year smolts. It is vital from the fish farmer's point of view to segregate potential first year smolts and reestablish them in other tanks. The first year smolts are gradually acclimatised to sea water before being transferred to sea pens in June. The smolts are very sensitive to handling at this time because of their high metabolic rate, general activity and nervousness, so great caution is required in order to prevent a high mortality rate upon transfer.

In the sea pens growth is rapid until about November when it starts to decline with the falling water temperatures and it remains low over the winter period.

Around March the growth rate picks up again and the fish may double their weight before the grilse "harvest" in late June or July. Regular feeding is interrupted just before this, to allow their guts to empty. The weight of the grilse may range from 2 to 6 lb at harvest time. Late July is chosen for harvesting when the fish are showing early signs of sexual maturity; maturation leads to a deterioration in their quality.

The remaining stock which have not yet reached the grilse stage, are left to feed and harvested as "salmon" from Christmas to the grilse harvest, although the majority are between early February and the end of April. The weight of these "salmon" ranges from 5 to 9 lbs. Naturally, it is in the fish farmer's interest to keep the feeding time to a minimum, with the cost of feed taking up a large part of the operating costs. The aim is to produce bigger fish in the same or less growth time.

The fish are fed a compound feed, based entirely on dry ingredients, in the form of pellets. The diet, ideally, resembles the natural one, with added proteins, vitamins and minerals. Pigments required for flesh and skin colour, which the fish obtains naturally in its marine diet, are also added. However, much effort is still being directed towards improving the farmed salmon diet in terms of its composition, texture, colour, size and shape and in establishing optimum feeding rates. Feed intake in high summer may be up to 3% body weight per day and in winter down to 0.4%. It depends both on metabolic rate, which is itself directly proportional to temperature, and the size of the fish.

Every attempt is being made by the salmon farmer to produce a fish of equal or superior "quality" to its wild counterpart. As well as being of obvious financial benefit to the fish farmer, in the long term the successful production of farmed salmon could have importance in the conservation of the species in this country. Hatchery reared alevins, parr and smolts are already used to restock rivers in Scotland: eyed eggs on the River Dee (Aberdeen), smolts in the North Esk (Montrose). In Eire, whole river systems are maintained by hatcheries producing smolts.

3. Objectives

The purpose of this investigation was to determine the lipid composition - in terms of component lipids and component acids - of salmon at various stages in their life cycle, in order to see if there were any significant changes which might be of value in the control of the diet of farmed fish or in other aspects of their husbandry. To this end, lipids from three sites - flesh, liver and gonads - have been examined from the following groups of fish.

	months in sea water	sex (no.)	farmed/wild
A	12	F (3)	farmed
A'	12	M (3)	farmed
B	18	F (2)	farmed
C*	18	F (2)	farmed
D	12	F (1)	wild
D'	12	M (1)	wild
E	24	F (1)	wild

* this group were sexually mature, all others were sexually immature

The lipids were also analysed from a sample of the diet fed to the farmed fish.

The results of these analyses made it possible to make several interesting comparisons of component lipids and component acids between (i) lipid site, (ii) female and male fish, (iii) farmed and wild fish, (iv) mature and immature fish, (v) immature farmed fish after 12 and 18 months in sea water, (vi) individual farmed salmon which had spent the same length of time in sea water (12 months) and (vii) farmed fish lipids and dietary lipids.

4. General procedures

Total lipids were extracted from each tissue by standard procedures and separated by preparative TLC. In one chromatographic system, individual neutral lipids, triacylglycerols, cholesterol and cholesterol esters, were separated from phospholipids (not recovered). Using another portion of the total lipid extract and a second solvent system individual polar lipids, phosphatidyl choline, phosphatidyl ethanolamine, cardiolipin and phosphatidyl inositol, were separated from neutral lipids (not recovered).

Neutral lipids were estimated by gravimetry and polar lipids by estimation of the P content of an aliquot of the extracted lipid solution from TLC. The methyl esters of each lipid were then obtained by transesterification and examined by GLC. Chromatographic data (retention time and height of peak) provided equivalent chain length, relative retention time and weight per cent with the aid of a computer programme (each GLC trace contained 20 to 30 peaks). Each chromatogram was run in duplicate. The detailed results are available on file but are not reproduced in this thesis. Results have been grouped in an appropriate manner and are presented as

mean values and standard deviations (calculated by the N method of statistical analysis) eg. palmitic acid mean 9.7% and standard deviation 1.9%, is written 9.7% (1.9).

The constraints imposed by time and the availability of appropriate samples has necessarily restricted the number of samples analysed during this project. A few results were discarded because of accidental contamination of the samples. This problem was quickly identified and experimental procedures were modified to eliminate this problem. For a study of this nature, ideally one should have analysed larger numbers of farmed and wild salmon over a period of several years in order to obtain "statistically significant" results. However, in this project it was hoped, from the limited number of samples available, to identify general trends upon which perhaps, further long-term experimental projects could be based.

5. Identification of fatty acids

The fatty acids present in salmon lipids have been identified (as their methyl esters) mainly on the basis of their gas chromatographic behaviour, but these conclusions are confirmed by some additional experiments involving silver ion chromatography and mass spectrometry.

Acids are usually identified from their retention behaviour on the basis of their equivalent chain length (ECL). On the polar columns used in this study, polyene esters are eluted later than their saturated analogues. Polar columns vary in polarity and change over a period of time, so that ECL values do not remain constant, but tend to decrease with increasing age of the column. Jamieson[67] has published useful data which relates the ECL of the more common polyene esters to that of α-linolenate in the range 19.5 to 21.0 . However, in the present studies α-linolenate is not a convenient reference peak. It is frequently small and may overlap with the peak for 20:1 ester, with the result that the ECL is not very accurate. It is better to use the more easily recognisable 20:5 (n-3) or 22:6 (n-3) esters as a way of finding the correct entries in Jamieson's tables. Despite this, it was sometimes

difficult to identify some of the smaller peaks with certainty and some peaks were not identified.

The relative retention time was found to give a less variable index and this often provided useful guidance when conclusions based on ECL were unclear. Relative retention times are frequently quoted with respect to 16:0 or 18:0, but this is unsatisfactory for several reasons. Such esters elute very early compared with the late running peaks such as 22:6 (n-3) and better results follow when the reference peak is nearer the middle of the chromatogram. The standard should also be easily recognised as a consistently dominant peak and if it is polyunsaturated it will show less variation with respect to the retention behaviour of other polyene esters with changing polarity of the column. 20:5 (n-3) served very satisfactorily in this connection.

In addition to the difficulty in recognising a few minor peaks, the major problem results from the overlap of C_{20}, C_{22} and C_{24} monoene esters with polyenes of shorter chain length. Typical overlapping peaks included 20:1 with 18:3 n-3, 22:1 with 20:4 n-3 or 20:4 n-6 and 24:1 with 22:5 n-6. The presence of each of these components can be demonstrated by Ag^+ chromatography or by mass spectrometry, but their relative proportions could not always be determined.

Silver ion chromatography separates natural long-chain esters mainly on the basis of the number of their double bonds (and configuration) although chain length and double bond positions have minor effects. A sample of methyl esters from salmon lipids was separated into several bands and each was examined by gas chromatography. In this way saturated esters, monoene esters etc were concentrated and then identified with the following results.

	major components	minor components
saturates	14:0, 16:0, 18:0	
monoenes	16:1, 18:1, 20:1	22:1, 24:1
dienes	18:2 (n-6)	16:2, 20:2
trienes	18:3 (n-3), 20:3 (n-6)	18:3 (n-6)? 20:3 (n-3)
n-3 polyenes	20:5, 22:5, 22:6	18:4, 20:4
n-6 polyenes	20:4	22:4, 22:5
other polyenes	-	16:4

GC-MS was carried out on several methyl ester samples, with the assistance of the Unilever Colworth Research Laboratory, in an attempt to prove or disprove (or add further information to) the provisional identifications made by the above methods. GC-MS is a sensitive analytical technique for examining the structure of fatty acid methyl esters, although it suffers from the drawback of being unable to pinpoint exactly the double bond position(s) in unsaturated fatty alkyl chains. Only tentative identifications, therefore, can be made of the more highly unsaturated members of the n-3 and n-6 polyene acid families.

Saturated and to a lesser extent monoenoic fatty acids, give rise to characteristic fragment ions in the mass spectrometer which have been well documented by McCloskey[68]. Besides the molecular ion (M^+), saturated fatty acids commonly show intense fragment ions at m/e M-31 (loss of $\cdot OCH_3$), at M-29 and M-43 (as a result of skeletal rearrangement of the chain and one hydrogen, followed by loss of an ethyl and a propyl radical respectively) and at 87, 143, 199 and 255 (prominent members of the series

$$CH_2{=}CH{-}(CH_2)_n{-}\overset{+OH}{\overset{\|}{C}}{-}OCH_3\ ,$$

formed by initial

hydrogen abstraction by the molecular ion, followed by rearrangement and cleavage of the long chain). The base peak usually occurs at m/e 74

$(CH_2{=}\overset{\overset{+\cdot OH}{|}}{C}{-}OCH_3)$, resulting from McLafferty rearrangement of the methyl ester.

Monoenes tend to show the most intense fragment ions at m/e M-31 (loss of $\cdot OCH_3$), M-32 (loss of CH_3OH), M-74 (McLafferty rearrangement, but with the positive charge remaining on the unsaturated alkyl fragment), M-116 and 74 (McLafferty) . Less intense hydrocarbon ions may also be observed.

The polyene acid fragmentation patterns are more complex, particularly in the densely populated lower m/e range and it is difficult to make any positive structural analyses on the basis of characteristic fragment ions, besides M^+, M-31 and M-32 ion fragments.

Listed on page 42 are the major distinguishable fatty acids and the m/e values of the dominant fragment ions upon which the assignments were based.

Fatty Acid	m/e value of characteristic fragment ions
16:0	270 (M^+), 241 (M-29), 239 (M-31), 227 (M-43), 143, 87*, 74
16:1	236 (M 32), 194 (M-74), 97, 83, 69, 57*, 55
18:0	298 (M^+), 269 (M-29), 267 (M-31), 255 (M-43), 199, 143, 87, 74*
18:1	296 (M^+), 265 (M-31), 264 (M-32), 222 (M-74), 180 (M-116), 139, 125, 111, 97*, 87
18:2	294 (M^+), 263 (M-31), 262 (M-32), 178 (M-116), 95, 81, 67*
20:0	326 (M^+), 283 (M-43), 143, 87, 74*
20:1	293 (M-31), 292 (M-32), 250 (M-74), 208 (M-116), 87, 74, 69, 55*
20:2	322 (M^+), 291 (M-31), 95, 81, 67*
20:3	320 (M^+), 289 (M-31), 95, 81, 67
20:3 (n-3?)	320 (M^+), 264 (M-56), 108, 95, 79*, 67
20:5	316 (M^+), 108, 95, 79*, 67
22:6	311 (M-31), 108, 95, 79*, 67

* indicates base peak

The existence of 20:3 (n-3) was suggested by the characteristic fragment ions shown above.

A series of peaks were usually observed in the GLC trace between 16:1 and 18:0, usually with ECL values > 17.0 .

GC-MS indicated the existence of the 17:0 ester [m/e 284 (M^+) 259 (M-31), 241 (M-31), 87, 74] and, in some cases, the possibility of 17:1, 17:0 branched and 18:0 branched esters.

From GLC chromatographic data, Ag^+ TLC and GC-MS results, the following general conclusions can be drawn about the fatty acids present.

(1) The saturates included 14:0, 16:0, 17:0, 18:0 and 20:0.

(2) The recognisable monoenes were 16:1, (17:1?), 18:1, 20:1, 22:1 and 24:1.

(3) All the members of the n-6 polyene acid family usually present in marine lipids were found: 18:2, 18:3, 20:3, 20:4, 22:4 and 22:5

(4) The n-3 polyene acids identified were 18:3, 18:4, (20:3?), 20:4, 20:5, 22:5 and 22:6.

Possibly present were 16:2, 16:4, 17:0 br and 18:0 br.

6. Dietary lipids (farmed salmon)

Total lipids were extracted from a portion of the dry (pelleted) diet (see Experimental, 2(ii)), separated into their component neutral and polar lipids in two separate TLC systems and quantitated. The major lipids were then transesterified and their fatty acid methyl esters examined by GLC.

The following results were obtained.

(i) Component lipids (% total lipids)

Lipid	
% lipid/~~100~~ diet	20.9
neutral lipids	
TG	70.1
CE	3.3
CHOL	5.4
OTHERS (DG, FFA etc)	10.2
sub-total	89.0
polar lipids	
PC	6.9
PE	1.2
CL	~~0.6~~
*PI/PS/SM	~~2.3~~
sub-total	11.0

* > 70% PI

Food consumed in nature by fish is mainly protein and fat, with little carbohydrate. Rapidly growing fish can use protein to build and maintain body tissue and fat

to provide energy. This is the case with (Atlantic) salmon at sea, where rapid weight gains occur, so high fat levels are desirable for those kept in captivity during the sea water stages of life. Fat at a level of approximately 21% of the diet produces optimum growth in farmed (Atlantic) salmon and no pathological conditions caused by fatty infiltration of the liver (a danger when feeding higher fat levels) have been observed.

(ii) Component acids of dietary lipids

The component acids of the major lipids present in the diet are listed in Table 2. All the acids of the four categories (saturates, monoenes, n-6 and n-3 polyenes) usually also identified in the methyl ester analyses (of the fatty acids) of the lipids from fish tissues, have been listed.

The most striking observation is the very high levels of both linoleic (18:2 n-6) and linolenic (18:3 n-3) acid in the major lipid constituent of the diet, triacylglycerols. The minor lipid constituents, phosphatidyl cholines, phosphatidyl ethanolamines and cardiolipins, also exhibit high levels of 18:2 n-6, but lower levels of linolenic acid.

The lipid components of the diet are a mixture of vegetable oils (rich in 18:2 n-6), linseed oil (rich in 18:3 n-3), and fish meal(s) (high content(s) of n-3 polyenes, particularly 20:5, 22:5 and 22:6). This probably accounts for the high levels of linoleic and linolenic observed in the diet triacylglycerols. This mixture of fats in the diet adequately satisfies the essential fatty acid requirements of the fish (18:2 n-6 and 18:3 n-3), while providing the high n-3 polyene acid content which salmon obtain from their natural diet in the wild.

Table 2. Component acids (% wt) of dietary lipids

Acid	TG	CE	PC	PE	CL
Saturates					
14:0	1.7	1.5	1.0	3.1	-
16:0	8.8	4.4	16.1	20.9	5.8
18:0	2.2	1.0	2.2	3.2	6.8
Monoenes					
16:1	3.3	1.9	2.7	2.3	3.5
18:1	16.5	12.8	15.3	10.7	9.4
20:1	5.8	2.2	⌊3.2⌋	2.3	⌊10.3⌋
22:1	[a] ⌈4.5⌉	1.8	0.6	0.9	2.3
24:1	⌈0.9⌉	-	⌈2.8⌉	⌊2.2⌋	-
n-6 polyenes					
18:2	17.4	5.0	22.4	23.4	15.7
18:3	-	-	-	-	-
20:3	0.1	0.3	0.1	0.3	3.0
20:4	0.4	5.1	2.1	1.7	1.3
22:4	0.6	0.6	0.2	0.5	3.6
22:5	⌈0.9⌉	0.8	⌈2.8⌉	⌊2.2⌋	0.2
n-3 polyenes					
18:3	11.0	1.9	⌈3.2⌉	5.0	⌊10.3⌋
18:4	-	0.6	0.2	0.3	1.5
20:4	[a] ⌈4.5⌉	1.3	0.6	0.5	1.8
20:5	6.3	17.1	8.4	4.2	7.1
22:5	1.7	1.3	0.9	1.0	1.0
22:6	16.3	37.3	19.9	13.7	17.1
others	2.5	3.1	1.3	3.8	9.6

a ⌊ ⌋ indicates overlapping peaks

7. Lipids of farmed and wild salmon

The samples examined came from seven sources differing in sex, level of sexual maturity, period in sea water, and whether farmed or wild. Most of the fish were female and only one group were sexually mature (see "Objectives" page 33).

The following discussion refers to female fish only, unless otherwise stated.

(i) Component Lipids (Table 3)

(a) Total lipid

The proportion of lipid extracted from the tissue (wet and containing some of the chloroform-methanol solvent in which all samples were stored) varied from 1-7%. No clear pattern emerges, but there is a tendency for the liver and gonads to be richer in lipid than the flesh and for the younger fish (12 months in sea water) to have a lower proportion of lipid than the older fish (18 and 24 months in sea water). There is little difference in the figures for wild and farmed fish, except that the latter seem to have less gonad lipid.

Table 3. Component Lipids (% Total Lipids)

	FLESH							LIVER							GONADS				
	A	A'	B	C	D	D'	E	A	A'	B	C	D	D'	E	A	B	C	D	E
no. of samples	(3)	(3)	(2)	(2)	(1)	(1)	(1)	(2)	(2)	(2)	(2)	(1)	(1)	(1)	(6)	(2)	(2)	(1)	(1)
% lipid/ tissue (wet)	1.1	1.1	5.7	3.0	1.0	1.5	4.4	2.9	2.8	4.8	4.4	3.0	12.7	5.4	0.9	2.2	5.0	6.8	5.7
neutral lipids																			
TG	62.9	64.7	77.6	66.4	59.4	73.7	83.1	8.0	7.9	36.9	7.7	12.2	66.9	42.2	16.6	22.5	43.7	62.1	51.1
CE	1.7	0.2	1.4	1.6	1.7	0.7	1.0	2.8	4.6	7.5	5.3	7.6	8.7	13.6	12.0	16.3	1.9	5.5	7.7
CHOL	3.0	3.2	2.5	2.4	2.5	1.4	1.2	3.8	5.0	5.7	4.1	5.5	2.8	3.6	6.2	8.0	3.5	3.6	4.7
others	2.6	2.0	3.5	5.7	0.9	4.2	2.5	12.6	10.6	4.7	5.5	10.5	3.0	2.9	10.9	15.1	1.7	2.0	4.9
(subtotal)	70.2	70.1	85.0	76.1	64.5	80.0	87.8	27.2	28.1	54.8	22.6	35.8	81.4	62.3	45.7	61.9	50.8	73.2	68.4
polar lipids																			
PC	19.1	20.8	9.1	13.9	24.3	10.9	8.2	46.7	42.9	26.7	50.0	31.4	13.0	25.5	29.9	24.7	37.6	20.2	24.1
PE	5.9	4.8	3.4	6.2	5.7	3.5	2.2	13.1	13.5	10.8	14.9	4.7	2.8	6.2	11.5	7.6	3.7	3.2	2.5
CL	0.9	2.2	1.0	1.1	1.2	2.6	0.4	3.0	2.6	2.0	2.0	2.6	0.4	4.9	0.7	1.5	0.3	0.3	0.7
PI/PS/SM	3.9	2.1	1.5	2.7	4.3	3.0	1.4	10.0	12.9	5.7	10.5	25.5	2.3	1.1	12.2	4.3	7.6	3.1	4.3
(subtotal)	29.8	29.9	15.0	23.9	35.5	20.0	12.2	72.8	71.9	45.2	77.4	64.2	18.6	37.7	54.3	38.1	49.2	26.8	31.6

(b) Neutral and polar lipids in flesh, liver and gonads (male and female)

Neutral lipids are highest in the flesh (mean 76%, range 65-88%), lower in the gonads (mean 60%, range 46-73%), and least in the liver (mean 45%, range 23-81%). Since the balance is made up of polar lipids, these reach the highest levels in the liver (mean 55%, range 19-77%) (see Table 4).

The major neutral lipid, triacylglycerol, is accompanied by cholesterol, cholesterol esters and minor amounts of diacylglycerols and free fatty acids. There are less triacylglycerols in the liver and gonads than in the flesh lipids, but more of the other neutral lipids (see Table 3).

In common with other marine fish, the dominant polar lipid present is phosphatidyl choline with lesser amounts of phosphatidyl ethanolamine, cardiolipin and a group of lipids containing phosphatidyl inositol, phosphatidyl serine and sphingomyelin, usually dominated by phosphatidyl inositol ($>$ 70%). For the most part these have the highest concentration in the liver lipids and the lowest in the flesh lipids.

Table 4. Component Lipids (% Total Lipids)

Mean and Range

Lipid Class	Flesh		Liver		Gonads	
neutral lipids	Mean	Range	Mean	Range	Mean	Range
TG	69.7	59.4-83.1	26.0	8.0-66.9	39.2	16.6-62.1
CE	1.2	0.2- 1.7	7.2	2.8-13.6	8.7	1.9-16.3
CHOL	2.3	1.2 - 3.2	4.4	2.8- 5.7	5.2	3.5- 8.0
others	3.0	0.9 -5.7	7.1	2.9-12.6	6.9	1.7-10.9
(sub-total)	76.2	64.5-87.8	44.7	22.6-81.4	60.0	45.7-73.2
polar lipids						
PC	15.2	8.2-19.1	33.7	13.0-50.0	27.3	20.2-37.6
PE	4.6	2.2-6.2	9.4	2.8-14.9	5.7	2.5-11.5
CL	1.3	0.4-2.6	2.5	0.5-4.9	0.7	0.3-1.5
PI/PS/SM	2.7	1.4-4.3	9.7	1.1-25.5	6.3	3.1-12.2
(sub-total)	23.8	12.2-35.5	55.3	18.6-77.4	40.0	26.8-54.3

(c) 12 and 18 month farmed fish

Farmed fish contain a higher proportion of lipid the longer they have been in sea water, in all three sites examined. In all cases there is an increase in the proportion of triacylglycerols, balanced by a decrease in the proportion of polar lipids, especially PC and PE. There is, however, a slight increase in the amount of phospholipid, since the lower proportion of these lipids is balanced by the higher proportion of lipid in the tissues.

	Flesh		Liver		Gonads	
months in sea water	12	18	12	18	12	18
total lipid (%)	1.1	5.7	2.9	4.8	0.9	2.2
TG (%)	63.0	78.0	8.0	37.0	17.0	23.0
PC + PE (%)	25.0	13.0	60.0	38.0	41.0	32.0

(d) Mature and immature

The mature farmed fish (18 months in sea water) have less lipid in the flesh, about the same in the liver, and more in the gonads compared with immature fish (same period in sea water). The mature group also contain less neutral lipids, but more PC and (except for the gonads) PE.

(e) Farmed and wild

The wild female fish which had been in sea water for approximately 1 year and 2 years respectively are very different in the composition of their flesh and liver lipids (gonad lipids are more similar) and it is difficult to compare these widely differing specimens with those obtained from farmed fish. Comparing farmed and wild fish both of which had been in sea water for about 1 year, the flesh and liver lipids are not markedly different, but this did not hold for gonad lipids. The much greater quantity of gonad lipid from the wild fish (6.8 vs 0.9%) is reflected in a much higher TG (62.1 vs 16.6%) content. The other components are at a lower level in the wild fish, but this is compensated for when the amount (as opposed to the proportion) of lipid is considered.

(f) Male and female

The small number of male fish examined limits the comparison which can be made of male/female lipid composition. The three male 12 months-in-sea farmed fish produce flesh and liver lipids which do not differ in any marked manner from those of three female fish from a similar environment.

On the other hand, the single male wild fish (12 months in the sea) is very different in its lipids from the single wild female fish which had spent a similar time in the sea.

(ii) Component acids of each lipid class

The component acids of each major lipid class of farmed and wild salmon have for convenience been grouped into saturates, monoenes, n-6 and n-3 polyenes. Only the major components of each of these groups of acids have been tabulated, namely 16:0 and 18:0, 16:1, 18:1 and 20:1, 18:2 n-6 and 20:4 n-6 and 20:5, 22:5 and 22:6 n-3. The sum of these acids is usually at least 80% and in some cases greater than 90% of the total acids.

The component fatty acids of the individual salmon lipids will be discussed initially in terms of the variations of the levels of these among the three lipid sites (flesh, liver and gonads) and between farmed and wild fish. Differences in the levels of acids for mature and immature fish and male and female fish are discussed for individual salmon lipids after the completion of these initial comparisons.

(a) Triacylglycerols (Table 5).

The triacylglycerols from the seven categories of salmon examined contain , 16:0 and 18:0 as the major saturated acids. Palmitic acid [9.7% (1.9)] is the dominant member of this group and its value varies little between male and female fish, farmed and wild, mature and immature, or among the three lipid sites (flesh, liver and gonads).

The major monoene acid is 18:1, accompanied by lower levels of 16:1, 20:1 and 22:1. In flesh lipids, the 16:1 [6.5% (1.5)] and 18:1 [23.4% (2.2)] acids vary little among the various categories of fish, but in liver these levels are slightly higher and somewhat more variable [16:1, 7.0% (2.1); 18:1, 30.7% (5.8)]. The gonads have lower monoene levels [16:1, 1.4% (0.5); 18:1, 18.2% (3.6)] than the liver and the flesh.

Only linoleic (18:2) and arachidonic (20:4) among the n-6 polyene acids are consistently present at significant levels ($>1\%$). Arachidonic occurs at low levels (1-6%) among the various categories whereas the value for linoleic shows interesting variations, ranging from 1-18%, with consistently higher values being shown for farmed fish compared to wild ones,

Table 5. Component acids (% wt) of Triacylglycerols

		FLESH											LIVER						GONADS						
	[b]Total	A		A'			B		C		D	D'	E	A	B		C	D	D'	E	A	B		C	D	E
Saturates	(13.2)	(13.8)	(12.6)	(13.0)	(15.0)	(17.2)	(12.5)	(14.8)	(14.3)	(12.0)	(12.6)	(16.1)	(12.8)	(13.7)	(10.3)	(11.4)	(15.4)	(8.6)	(9.5)	(10.0)	(9.8)	(5.3)	(12.0)	(10.3)	(11.9)	(9.6)	(8.6)	(10.8)	(11.1)
16:0	10.1	10.5	9.4	10.0	12.2	13.1	9.8	11.7	10.7	9.3	10.0	13.2	10.7	10.2	7.1	8.8	12.0	7.0	7.8	9.4	6.7	4.8	10.0	9.5	10.5	8.1	7.1	10.0	10.3
18:0	3.1	3.3	3.2	3.0	2.8	4.1	2.7	3.1	3.6	2.7	2.6	2.9	2.1	3.5	3.2	2.6	3.4	1.8	1.7	0.6	3.1	0.5	2.0	0.8	1.4	1.5	1.5	0.8	0.8
Monoenes	(37.8)	(35.6)	(37.6)	(35.2)	(39.1)	(41.0)	(34.1)	(37.4)	(37.3)	(36.1)	(42.0)	(36.7)	(40.3)	(39.6)	(38.1)	(45.3)	(57.5)	(35.0)	(35.0)	(54.1)	(40.6)	(48.7)	(26.6)	(23.7)	(25.6)	(33.7)	(32.7)	(24.9)	(23.7)
16:1	5.9	5.2	5.1	6.2	9.4	6.3	8.3	7.8	5.7	7.1	4.4	4.6	8.3	6.3	3.6	8.0	10.0	5.2	6.0	8.1	5.4	10.2	5.6	4.8	6.0	8.1	8.7	8.1	7.7
18:1	25.2	22.2	23.3	23.1	24.5	27.6	18.8	21.9	26.2	22.6	25.5	22.6	21.3	27.3	24.8	29.5	41.0	27.6	26.9	41.1	27.3	31.1	17.3	16.6	17.8	24.3	22.7	14.7	13.9
20:1	[a] [6.7]	[8.2]	[9.2]	[5.9]	[5.2]	[7.1]	7.0	7.1	5.4	6.4	[12.1]	[9.5]	10.7	[5.9]	[9.7]	[7.8]	6.5	[2.2]	2.1	[4.9]	[7.9]	7.4	[3.7]	2.3	1.8	1.3	1.3	[2.1]	2.1
n-6 polyenes	(17.0)	(18.5)	(20.8)	(17.4)	(18.3)	(17.0)	(15.9)	(10.2)	(14.7)	(13.0)	(1.4)	(9.2)	(1.9)	(18.1)	(24.2)	(7.5)	(3.2)	(13.3)	(12.2)	(6.5)	(5.5)	(2.7)	(12.1)	(9.0)	(7.5)	(14.3)	(13.4)	(5.3)	(3.1)
18:2	15.8	14.7	16.7	14.1	16.1	13.6	10.5	9.4	13.5	12.2	0.9	0.8	1.9	15.6	17.9	6.3	3.2	9.3	8.6	3.9	1.2	1.7	11.0	6.7	5.7	13.0	12.0	3.1	0.8
20:4	1.2	3.8	[4.1]	[3.3]	[2.2]	[3.4]	5.4	0.8	1.2	0.8	0.5	8.4	-	2.5	6.3	1.2	-	4.0	3.6	[2.6]	[4.3]	1.0	1.1	2.3	1.8	1.3	1.3	[2.2]	2.3
n-3 polyenes	(20.2)	(21.7)	(20.4)	(25.0)	(19.3)	(16.0)	(22.6)	(21.1)	(22.4)	(23.7)	(29.7)	(29.7)	(26.1)	(19.8)	(19.5)	(22.6)	(18.2)	(32.4)	(31.3)	(20.5)	(33.7)	(29.4)	(41.5)	(54.4)	(46.1)	(31.4)	(34.1)	(51.2)	(53.2)
20:5	4.4	5.6	5.2	4.8	4.6	4.7	6.9	5.9	5.0	4.8	6.5	7.0	8.4	5.2	6.0	4.7	7.8	9.9	7.8	9.4	8.7	10.9	7.8	13.5	11.0	7.4	7.7	13.4	13.1
22:5	2.4	2.9	3.2	2.7	2.7	2.4	3.3	2.9	4.7	4.6	5.1	3.3	3.9	2.6	2.7	4.4	2.1	5.2	5.1	3.9	7.0	8.8	[2.1]	4.9	3.8	4.9	4.4	4.3	4.3
22:6	13.4	13.2	12.0	17.5	12.0	8.9	12.4	12.3	12.7	14.9	12.7	19.4	13.8	12.0	10.8	13.5	8.3	17.3	21.4	7.2	18.0	9.7	31.6	36.0	31.2	19.1	22.0	33.5	35.8

a see footnote Table 2

b totals refer only to those acids listed

	Flesh		Liver		Gonads	
	farmed	wild	farmed	wild	farmed	wild
18:2 n-6	13.7(2.3)	1.2(0.5)	10.2(5.1)	2.3(1.2)	9.7(2.9)	2.0(1.2)

The farmed fish are fed a comparatively linoleic-rich diet, for example, 17% of this acid is present in the triacylglycerols. This probably accounts for the difference from wild fish which have a lower lipid intake and less n-6 polyene acids in their diet. The marine zooplankton Euphausia pacifica, upon which sockeye salmon [Oncorhynchus nerka] feed, has a content of linoleic plus linolenic acid of only 4% of the total fatty acids of its total neutral lipid components[69].

The level of the major n-3 polyene acids, 20:5, 22:5 and 22:6, is similar in flesh and liver, but is significantly higher in the gonads, particularly in the proportion of 22:6 present.

	Flesh	Liver	Gonads
20:5 + 22:5 + 22:6	22.5(3.3)	25.6(6.3)	44.5(8.5)
22:6	13.5(2.5)	13.1(4.6)	29.9(6.2)

No significant differences are observed for these acids between farmed and wild fish.

The immature (B) and mature (C) fish show several interesting differences in their component acids. The immature fish have more monoene and less n-6 and n-3 polyenes in their liver compared with the mature fish, whereas in the gonads the immature fish have less monoenes, less n-6 polyenes and more n-3 polyenes.

	Flesh		Liver		Gonads	
	Mature	Immature	Mature	Immature	Mature	Immature
a	37.6(0.6)	35.8 (1.6)	35.0(0.0)	51.4 (6.1)	33.2(0.5)	24.7 (1.0)
b	13.9(0.8)	13.1 (2.8)	12.8(0.6)	5.4 (2.2)	13.9(0.5)	8.3 (0.8)
c	23.1(0.6)	21.9 (0.8)	33.4(1.0)	20.4 (2.2)	32.8(1.4)	50.4 (4.2)

a. 16:1 + 18:1 + 20:1

b. 18:2 + 20:4

c. 20:5 + 22:5 + 22:6

There are differences between the levels of n-3 polyene acids (particularly in the proportion of 22:6) in the flesh and liver of male and female wild salmon (D' and D respectively). For both sites the male fish has the highest level of 22:6. The male fish, however, has a lower level of 18:1 (27.3%) in the liver than the female fish (41.1%).

	Flesh		Liver	
	Male	Female	Male	Female
20:5 + 22:5 + 22:6	29.7	24.3	33.7	20.5
22:6	19.4	12.7	18.0	7.2

It must be remembered that this is only a comparison between two single fish. No differences are observed among the levels of the four groups of acids (saturates, monoenes, n-6 and n-3 polyenes) between the male and female flesh of the farmed salmon (A' and A respectively).

(b) Cholesterol esters (Table 6)

The cholesterol ester content is usually a low proportion of the total lipids of marine fish. Also, their methyl esters are known to show wide variations in their levels of the "normal" range of observed fatty acids, sometimes accompanied by high levels of unusual fatty acids (eg. furanoid acids[1a]) when compared with those of the major lipid classes.

In the present study of the methyl esters of the cholesterol esters of Atlantic salmon (Salmo salar) an unexpectedly high content of arachidonic is sometimes observed in the flesh and liver of farmed salmon (A/A'). These abnormally large values for 20:4 (n-6) distort the range of values for the remaining saturated, monoene and n-3 polyene acids from these sites. No useful comparisons can therefore be made of the levels of the four main groups of fatty acids among the lipid sites, and between male and female fish, farmed and wild fish.

One comparison unaffected by these high levels of 20:4 (n-6) is between mature and immature fish. The immature fish have more n-3 polyenes, but less monoene

Table 6. Component acids (% wt) of Cholesterol esters

	FLESH					LIVER									GONADS			
	A [b]Total	A'	B	C	D'	A	A'	B		C		D	D'	E	A	C	D	E
Saturates	(7.2)	(1.9)	(17.6)	(17.9)	(16.8)	(19.1)	(10.2)	(30.7)	(8.0)	(8.1)	(14.9)	(11.4)	(10.6)	(5.9)	(17.5)	(9.4)	(17.1)	(3.4)	(2.9)
16:0	5.0	1.2	12.5	12.6	13.7	15.9	8.5	21.7	7.0	6.7	12.2	8.9	8.9	4.9	15.8	4.2	5.1	3.1	2.1
18:0	1.2	0.7	5.1	5.3	3.1	3.2	1.7	9.0	1.0	1.4	2.7	2.5	1.7	1.0	1.7	5.2	12.0	0.3	0.8
Monoenes	(9.4)	(3.7)	(28.1)	(32.7)	(27.4)	(8.5)	(17.6)	(21.0)	(34.2)	(38.9)	(18.2)	(23.7)	(21.9)	(24.8)	(26.8)	(19.8)	(17.0)	(26.8)	(18.3)
16:1	2.0	0.5	4.2	4.4	11.0	2.8	2.2	5.5	2.0	3.2	2.4	4.2	6.7	3.5	3.7	6.3	1.9	6.7	3.8
18:1	6.0	2.9	17.5	23.0	12.2	4.4	10.1	12.1	17.3	20.6	12.6	14.8	12.0	12.7	12.3	8.5	13.6	17.7	12.1
20:1	[a][1.4]	[0.3]	[6.4]	5.3	[4.2]	[1.3]	[5.3]	[3.4]	[14.9]	15.1	[3.2]	4.7	[3.2]	[8.6]	10.8	[5.0]	1.5	[2.4]	2.4
n-6 polyenes	(68.0)	(82.1)	(5.4)	(11.1)	(7.4)	(47.8)	(42.5)	(10.0)	(4.1)	(4.8)	(7.9)	(7.7)	(8.4)	(8.0)	(5.4)	(8.9)	(13.7)	(3.9)	(4.2)
18:2	2.3	0.6	4.1	8.8	0.5	3.2	5.6	5.8	2.1	2.8	3.7	5.5	2.6	2.0	1.0	6.3	11.5	1.6	1.6
20:4	65.7	81.5	1.3	2.3	6.9	44.6	36.9	4.2	2.0	2.0	4.2	2.2	5.8	[6.0]	4.4	2.6	2.2	[2.5]	2.6
n-3 polyenes	(9.0)	(3.1)	(33.1)	(25.7)	(28.7)	(14.0)	(18.7)	(24.3)	(30.9)	(32.9)	(43.6)	(35.2)	(23.0)	(37.8)	(24.8)	(20.7)	(40.7)	(58.2)	(63.3)
20:5	2.3	0.6	10.3	6.6	6.4	2.6	4.0	7.2	11.7	10.0	12.8	8.5	11.1	11.5	9.6	3.4	9.7	18.2	18.9
22:5	0.8	0.4	2.3	2.4	1.3	0.6	1.7	1.8	4.7	5.5	2.2	3.2	3.0	10.7	8.3	[0.7]	[2.6]	5.5	5.2
22:6	5.9	2.1	20.5	16.7	21.0	10.8	13.0	15.3	14.5	17.4	28.6	23.5	8.9	15.6	6.9	16.6	28.4	34.5	39.2

a see footnote Table 2

b totals refer only to those acids listed

and n-6 polyene acids in their flesh compared with mature fish, whereas in the liver the immature fish have more monoenes, but less saturates, n-6 and n-3 polyenes.

	Flesh		Liver	
	Mature	Immature	Mature	Immature
16:0 + 18:0	17.9	17.6	13.2 (1.7)	8.1 (0.1)
16:1 + 18:1 + 20:1	32.7	28.1	21.0 (2.7)	36.6 (2.3)
18:2 + 20:4	11.1	5.4	7.8 (0.1)	4.5 (0.3)
20:5 + 22:5 + 22:6	25.7	33.1	39.4 (4.2)	31.9 (1.0)

(c) Phosphatidyl cholines (Table 7)

Palmitic acid is the major saturated acid in the flesh [15.1% (2.5)], liver [13.2% (1.9)] and gonads [18.8% (4.8)]. Stearic acid is also present at lower levels (0.3-5.3%), but rises to 7-8% in the liver and gonad phosphatidyl cholines from mature fish. No substantial differences are noted between farmed and wild fish, male and female fish, nor from the different lipid sites.

The usual range of monoene acids (16:1-24:1) is present, with the C_{16} and C_{18} acids predominating. The content of 18:1 is notably higher in the liver [11.4% (2.4)] and gonads [12.7% (3.3)] than in the flesh [6.0% (2.2)], but no other differences of interests are noted.

Of the n-6 acids present in the phosphatidyl cholines, linoleic (0.3-6.2%) and arachidonic (0.9-5.1%) are the most important. No differences are apparent among the various categories of fish or among the lipid sites.

The n-3 acids are the major components in the phosphatidyl cholines. The three major members (20:5, 22:5 and 22:6) are slightly higher in the flesh [66.6% (5.2)] than in the liver [55.6% (5.9)] or the gonads [48.6% (6.7)]. This difference is also to be seen in the 22:6 levels: flesh [51.4% (6.1)], liver [38.0% (5.9)], and gonads [33.4% (5.6)].

Table 7. Component acids (% wt) of Phosphatidyl cholines

		FLESH												LIVER							GONADS					
	[b]Total	A		A'			B		C		D	D'	E	A	A'	B		C	D	D'	E	A	B		C	D	E
Saturates	(13.4)	(11.7)	(14.8)	(33.4)	(21.2)	(13.8)	(17.4)	(19.7)	(17.1)	(20.0)	(17.9)	(14.7)	(14.2)	(12.5)	(14.8)	(18.2)	(22.3)	(21.1)	(20.9)	(18.7)	(12.7)	(15.2)	(21.3)	(28.3)	(25.9)	(23.1)	(21.0)	(17.4)	(18.5)
16:0	12.5	11.2	14.5	31.0	17.7	10.9	16.7	18.6	16.2	17.7	17.4	14.3	13.8	10.5	12.6	14.7	17.0	13.2	12.9	14.0	10.8	13.0	20.2	27.2	24.2	14.7	14.0	15.1	16.4
18:0	0.9	0.5	0.3	2.4	3.5	2.9	0.7	1.1	0.9	2.3	0.5	0.4	0.4	2.0	2.2	3.5	5.3	7.9	8.0	4.7	1.9	2.2	1.1	1.1	1.7	8.4	7.0	2.3	2.1
Monoenes	(6.3)	(11.9)	(8.7)	(20.8)	(8.2)	(6.0)	(9.4)	(11.1)	(7.3)	(8.7)	(6.9)	(11.4)	(7.1)	(14.3)	(13.8)	(18.3)	(17.9)	(14.9)	(14.4)	(22.9)	(15.0)	(16.9)	(21.7)	(23.0)	(22.1)	(14.3)	(13.1)	(26.5)	(17.3)
16:1	-	6.0	3.1	6.6	2.0	-	2.0	2.3	1.5	1.7	1.4	4.3	2.6	3.4	2.6	1.5	2.5	1.2	1.3	3.3	3.5	4.7	8.3	4.1	4.0	1.4	1.6	15.6	4.2
18:1	4.9	4.6	4.2	12.6	5.2	5.3	6.5	8.0	5.0	6.1	4.9	6.3	4.1	8.4	8.6	13.6	12.7	12.4	11.0	16.3	9.8	9.8	12.4	17.9	17.4	11.8	10.1	8.7	10.7
20:1	[a][1.4]	[1.3]	[1.4]	[1.6]	[1.0]	[0.7]	[0.9]	[0.8]	[0.9]	0.9	[0.6]	[0.8]	0.5	[2.5]	[2.6]	[3.2]	2.7	[1.3]	2.1	[3.3]	[1.7]	2.4	[1.0]	[1.0]	0.7	1.1	1.4	[2.2]	2.4
n-6 polyenes	(4.7)	(3.7)	(3.2)	(5.3)	(4.9)	(4.2)	(3.7)	(4.4)	(4.0)	(3.7)	(1.4)	(6.1)	(1.0)	(8.7)	(6.8)	(5.1)	(7.2)	(5.4)	(4.9)	(2.4)	(2.2)	(2.4)	(4.8)	(3.9)	(5.3)	(5.1)	(5.9)	(1.9)	(2.4)
18:2	3.3	2.2	1.5	3.6	1.7	1.6	1.7	2.4	1.9	1.7	0.3	1.0	0.1	6.2	4.9	2.2	2.5	2.1	2.1	0.8	0.5	0.4	2.4	0.8	1.5	2.7	2.4	0.5	0.2
20:4	1.4	1.5	[1.7]	1.7	3.2	[2.6]	2.0	2.0	2.1	2.0	[1.1]	5.1	0.9	2.5	1.9	2.9	4.7	3.3	2.8	[1.6]	[1.7]	2.0	2.4	3.1	3.8	2.4	3.5	1.4	2.2
n-3 polyenes	(71.8)	(70.6)	(70.7)	(31.3)	(59.0)	(73.5)	(64.5)	(58.5)	(67.5)	(61.7)	(71.5)	(61.2)	(68.9)	(60.4)	(56.2)	(53.4)	(46.8)	(51.9)	(52.4)	(52.2)	(67.1)	(59.9)	(47.9)	(36.8)	(42.4)	(50.4)	(54.1)	(49.9)	(58.7)
20:5	10.0	10.8	12.0	5.7	9.8	13.6	17.2	15.9	15.8	10.9	14.9	10.4	12.4	10.3	9.8	16.9	13.2	10.4	8.3	17.1	19.2	16.5	11.7	11.4	11.1	9.0	10.4	12.0	14.6
22:5	1.5	2.2	2.6	1.1	2.3	2.6	3.6	3.3	[2.9]	2.6	2.2	2.1	1.3	2.1	4.0	4.2	3.4	4.6	3.9	4.6	4.1	5.1	1.8	2.6	[2.7]	5.9	4.6	4.3	4.1
22:6	60.3	57.6	56.1	24.6	46.9	57.3	43.7	39.3	48.8	48.2	54.4	48.7	55.2	48.0	42.4	32.3	30.2	36.9	40.2	30.5	43.8	38.3	34.4	22.8	28.6	35.5	39.1	33.6	40.0

a see footnote Table 2

b totals refer only to those acids listed

On the basis of two fish of each kind, some differences can be seen between the mature and immature fish. The 22:6 (n-3) acid level is lower for the immature fish at all three lipid sites compared to mature fish, although this trend is less marked for the total n-3 acids except for the gonads. The immature fish have higher monoene levels than the mature fish in the liver and gonads (the latter organ also having a higher saturates content for the immature fish).

	Flesh		Liver		Gonads	
	Mature	Immature	Mature	Immature	Mature	Immature
a	18.6 (1.5)	18.6 (1.2)	21.0 (0.2)	20.3 (2.0)	22.1 (1.0)	27.1 (1.2)
b	8.0 (0.7)	10.3 (0.9)	14.7 (0.3)	18.1 (0.2)	13.7 (0.6)	22.1 (0.1)
c	64.6 (2.9)	61.5 (3.0)	52.2 (2.0)	50.1 (3.3)	52.2 (1.8)	39.6 (3.9)
d	48.5 (0.3)	41.5 (2.2)	38.6 (1.6)	31.3 (1.0)	37.3 (1.8)	25.7 (2.9)

a. 16:0 + 18:0

d. 16:1 + 18:1 + 20:1

c. 20:5 + 22:5 + 22:6

d. 22:6

Differences exist between the levels of n-3 polyene acids in the flesh and liver of male and female wild salmon.

	Flesh		Liver	
	Male	Female	Male	Female
20:5 + 22:5 + 22:6	61.2	70.2(1.3)	67.1	56.1(3.8)

However, it must be appreciated that this is a single male fish and therefore differences can hardly be considered significant.

(d) Phosphatidyl ethanolamines (Table 8)

The major saturated acid is palmitic [7.5% (2.0)] which shows little variation among either the different categories of fish or the various sites from which the lipid was taken. Though usually present at slightly lower levels (2.5-4.6%) stearic acid reaches 8-10% in the liver and gonads of mature fish.

The 16:1 and 18:1 members predominate among the C_{16}-C_{24} monoene acids. The content of 18:1 is higher in the liver [12.0% (2.6)] and gonads [13.6% (1.0)] than in the flesh [7.0% (1.3)]. Palmitoleic acid is present at lower levels (0.8-2.1%) in farmed fish, but significantly higher proportions are found in the flesh and gonads of wild fish. No differences are observed for male and female fish or from mature and immature fish.

The major n-6 polyene acids present in phosphatidyl ethanolamines are linoleic (0.3-9.2%) and arachidonic (1.5-9.6%). Farmed fish tend to have higher levels of these two acids than wild fish in their liver and gonads.

	Flesh		Liver		Gonads	
	Farmed	Wild	Farmed	Wild	Farmed	Wild
18:2 + 20:4	5.0(0.8)	5.5 (4.3)	10.4 (3.6)	2.7	9.2 (1.5)	4.5

Table 8. Component acids (% wt) of Phosphatidyl ethanolamines

		FLESH									LIVER						GONADS					
	[b]Total	A	A'			B	C	D	D'	E	A	A'		B	C	E	B		C		D	E
Saturates	(10.6)	(9.9)	(22.0)	(5.9)	(11.4)	(13.2)	(13.3)	(11.4)	(12.6)	(14.1)	(10.2)	(10.2)	(11.4)	(12.4)	(13.9)	(9.0)	(12.1)	(12.6)	(13.3)	(15.8)	(14.7)	(5.9)	(9.8)
16:0	7.4	7.4	15.1	2.1	7.8	9.9	9.7	8.8	9.7	10.8	6.5	6.3	8.3	7.8	5.8	4.9	8.0	9.1	9.7	5.5	5.7	3.6	6.7
18:0	3.2	2.5	6.9	3.8	3.6	3.3	3.6	2.6	2.9	3.3	3.7	3.9	3.1	4.6	8.1	4.1	4.1	3.5	3.6	10.3	9.0	2.3	3.1
Monoenes	(10.7)	(8.5)	(14.4)	(14.1)	(9.5)	(11.7)	(9.0)	(27.0)	(19.6)	(9.6)	(11.4)	(13.0)	(12.1)	(23.6)	(17.5)	(15.7)	(23.2)	(16.0)	(17.8)	(18.8)	(16.9)	(60.1)	(17.4)
16:1	2.1	1.9	3.0	7.6	1.6	1.0	0.8	14.3	9.9	1.0	0.9	0.8	1.0	1.9	0.6	1.7	1.8	1.3	1.9	0.8	1.1	50.3	1.3
18:1	6.6	5.4	9.0	5.5	6.5	7.8	7.0	10.1	7.6	6.7	8.9	10.2	9.8	16.4	13.2	10.8	14.8	13.4	13.9	15.3	12.5	6.8	12.9
20:1	[a][2.0]	[1.2]	[2.4]	[1.0]	[1.4]	[2.9]	1.2	[2.6]	[2.1]	1.9	[1.6]	[2.0]	[1.3]	5.3	3.7	[3.2]	6.6	[1.3]	2.0	2.7	3.3	[3.0]	3.2
n-6 polyenes	(5.7)	(4.6)	(5.3)	(6.1)	(5.2)	(4.8)	(3.7)	(11.6)	(2.5)	(2.5)	(10.9)	(12.1)	(11.2)	(5.0)	(7.2)	(16.1)	(2.7)	(7.1)	(8.5)	(10.9)	(10.2)	(2.6)	(4.5)
18:2	3.6	2.6	3.4	2.2	3.4	2.7	2.2	2.0	0.3	0.3	6.8	6.7	7.0	1.8	1.8	9.2	0.7	2.5	3.3	3.6	2.4	0.7	1.3
20:4	2.1	[2.0]	1.9	[3.9]	1.8	2.1	1.5	[9.6]	2.2	2.2	4.1	3.4	4.2	3.2	5.4	6.9	2.0	4.6	5.2	7.3	7.8	1.9	3.2
n-3 polyenes	(61.3)	(69.5)	(38.3)	(70.4)	(62.5)	(59.5)	(67.0)	(42.5)	(56.3)	(64.1)	(63.3)	(58.5)	(60.3)	(52.2)	(53.2)	(40.2)	(56.1)	(55.5)	(52.7)	(46.7)	(46.2)	(28.2)	(63.3)
20:5	5.1	5.6	3.4	6.1	4.3	6.1	5.3	4.6	5.3	8.7	7.5	9.6	9.7	11.1	8.9	11.0	11.8	4.9	5.6	12.0	10.2	5.4	11.7
22:5	3.9	3.3	4.5	4.1	3.2	4.0	[4.3]	2.1	3.4	[3.0]	1.7	2.8	2.8	2.8	2.5	3.0	3.9	2.9	2.6	4.7	[4.1]	2.2	3.3
22:6	52.3	60.5	30.4	60.2	55.0	47.4	57.4	35.8	47.6	52.4	54.1	46.1	47.8	38.3	41.8	26.2	40.4	47.7	44.5	30.0	31.9	20.6	48.3

a see Footnote Table 2

b totals refer only to those acids listed

The n-3 polyene acids are the dominant components of the phosphatidyl ethanolamines. The three major members (20:5, 22:5 and 22:6) are not very different for the flesh [61.4% (8.0)], liver [54.8% (7.0)] and gonads [52.9% (6.3)]. The level of the most abundant n-3 acid, 22:6, also follows this trend: flesh [52.1% (7.3)], liver [42.1% (8.1)] and gonads [40.5% (7.9)]. The level of the three major n-3 acids tends to be higher in the flesh, but lower in the gonads of farmed fish compared to wild ones.

	Flesh		Liver		Gonads	
	farmed	wild	farmed	wild	farmed	wild
20:5 + 22:5 + 22:6	65.0(4.1)	54.3(8.9)	54.6(7.5)	56.1	50.3(4.0)	63.3

Differences can be observed between mature and immature fish in the total n-3 polyene acids, as can be seen in the following figures.

	Flesh		Liver		Gonads	
	Mature	Immature	Mature	Immature	Mature	Immature
a	67.0	59.5	46.7	52.2	46.5(0.3)	54.1(1.4)

a. 20:5 + 22:5 + 22:6

The immature fish have less n-3 polyene acids in the flesh compared to mature fish, but in the liver and gonads the immature fish have more n-3 polyene acids.

No significant differences are observed between male and female fish either farmed or wild, where these comparisons are available.

(e) Cardiolipins (Table 9)

Palmitic acid [11.8% (2.3)] is the major saturated acid and its value does not vary between male and female fish, mature and immature fish, farmed and wild fish or among the three lipid sites. Stearic acid usually occurs at lower levels (1.9-7.0%).

The major monoenes acids present are again 16:1 and 18:1 with 20:1, 22:1 and 24:1 in smaller amounts. The dominant monoene is 18:1 and its content is higher in the gonad lipids [19.6% (2.6)]. than in the liver [12.7% (3.2)] or flesh [15.3% (3.1)]. No significant differences are observed between farmed and wild fish or male and female fish.

Linoleic and arachidonic are the most prominent n-6 polyene acids. The levels of both linoleic (0.8-12.7%) and arachidonic (0.5-19.7%) exhibit considerable variations. However, the content of 18:2 tends to be greater for farmed than wild fish.

	Flesh		Liver		Gonads	
	farmed	wild	farmed	wild	farmed	wild
18:2 n-6	9.2(1.3)	0.8	8.5 (2.9)	1.0	5.6 (2.0)	0.9

The C_{20} and C_{22} polyenes are the major n-3 acids which are the largest components of the cardiolipins. The

Table 9. Component acids (% wt) of Cardiolipins

		FLESH									LIVER						GONADS			
	[b]Total	A		A'		B		C		E	A	A'	B	C		E	B		C	E
Saturates	(9.6)	(19.6)	(14.5)	(17.1)	(18.1)	(13.5)	(15.3)	(14.2)	(13.6)	(15.2)	(14.2)	(22.2)	(14.6)	(18.8)	(16.8)	(15.7)	(21.5)	(20.0)	(15.6)	(17.4)
16:0	5.5	14.2	11.0	12.3	13.3	10.3	11.6	11.2	9.6	12.4	9.1	11.6	11.3	11.8	10.9	13.8	16.1	13.3	11.0	15.5
18:0	4.1	5.4	3.5	4.8	4.8	3.2	3.7	3.0	4.0	2.8	5.1	10.6	4.3	7.0	5.9	1.9	5.4	6.7	4.6	1.9
Monoenes	(43.1)	(26.7)	(27.5)	(21.7)	(29.0)	(30.4)	(27.7)	(23.2)	(19.9)	(19.9)	(17.9)	(14.7)	(26.4)	(19.8)	(17.3)	(20.7)	(25.0)	(22.3)	(31.2)	(26.6)
16:1	5.0	4.8	4.2	5.5	6.3	6.2	5.1	4.0	3.2	3.8	2.8	1.6	4.1	3.1	8.3	4.3	3.8	4.0	5.6	5.9
18:1	8.6	17.8	16.6	13.1	18.2	18.3	18.4	15.2	14.0	12.5	10.0	10.7	18.5	14.6	9.0	13.3	19.5	17.1	23.9	18.0
20:1	[a] [29.5]	4.1	6.7	3.1	4.5	[5.9]	4.2	[4.0]	2.7	3.6	[5.1]	[2.4]	3.8	2.1	-	3.1	[1.7]	1.2	1.7	2.7
n-6 polyenes	(8.9)	(12.8)	(14.0)	(25.8)	(11.1)	(12.4)	(11.7)	(16.3)	(12.3)	(6.2)	(15.1)	(13.5)	(6.6)	(12.6)	(11.2)	(4.8)	(7.1)	(6.7)	(11.9)	(3.7)
18:2	8.4	9.6	11.4	6.1	9.0	9.3	10.0	9.5	9.8	0.8	12.7	10.3	4.1	8.3	6.9	1.0	4.4	4.1	8.4	0.9
20:4	0.5	[3.2]	[2.6]	[19.7]	[2.1]	[3.1]	1.7	6.8	2.5	5.4	2.4	3.2	2.5	4.3	4.3	3.8	2.7	2.6	3.5	2.8
n-3 polyenes	(18.8)	(27.0)	(29.7)	(21.3)	(29.8)	(34.1)	(30.8)	(31.1)	(35.3)	(42.8)	(36.9)	(35.3)	(39.0)	(31.0)	(21.0)	(42.6)	(31.0)	(30.2)	(22.3)	(43.1)
20:5	1.1	2.6	2.4	1.6	1.6	4.7	3.0	2.6	2.6	3.2	1.5	2.7	6.2	2.8	3.1	4.4	3.9	4.5	6.0	10.9
22:5	1.7	2.0	2.9	2.5	3.1	3.1	2.2	2.2	2.0	3.0	4.0	3.1	3.3	3.5	2.9	6.3	2.6	1.7	[2.9]	3.3
22:6	16.0	22.4	24.4	17.2	25.1	26.3	25.6	26.1	30.7	36.6	31.4	29.5	29.5	24.7	15.0	31.9	24.5	24.0	13.4	28.9

a see footnote Table 2

b totals refer only to those acids listed

content of these three acids (20:5, 22:5 and 22:6) is not very different for flesh [30.1% (6.5)], liver [34.3% (6.9)] and gonads [31.7% (7.4)] and this is also true for the 22:6 acid: flesh [25.0% (5.7)], liver [27.0% (5.8)] and gonads [22.7% (5.7)]. The farmed fish tend to have lower levels of these three acids (particularly 22:6) than the wild fish.

	Flesh		Liver		Gonads	
	farmed	wild	farmed	wild	farmed	wild
20:5 + 22:5 + 22:6	28.7(5.1)	42.8	32.6(6.4)	42.6	27.8(3.9)	43.1
22:6	23.8(4.4)	36.6	26.0(5.9)	31.9	20.6(5.1)	28.9

Several interesting differences occur between the component acids of immature and mature fish. The immature fish besides having more monoene in their flesh compared with mature fish, have more monoene, more n-3 polyenes and less n-6 polyene acids in their liver. In the gonads the immature fish have less monoene, less n-6 polyenes but more n-3 polyene acids compared to mature fish.

	Flesh		Liver		Gonads	
	Mature	Immature	Mature	Immature	Mature	Immature
a	22.2(1.2)	29.1(1.3)	18.6(1.3)	26.4	31.2	23.7 (1.3)
b	14.3(2.0)	12.1(0.3)	11.9(0.7)	6.6	11.9	6.9 (0.2)
c	33.2(2.1)	32.5(1.6)	26.0(5.0)	39.0	22.3	30.6 (0.4)

a. 16:1 + 18:1 + 20:1 b. 18:2 + 20:4 c. 20:5 + 22:5 + 22:6

Where comparisons are available between male and female fish (A/A'), the only observable difference is in the level of the 18:0 acid in the liver lipids. This is hardly significant, as there is only one fish of each type.

8. Observations

(i) Distribution of lipids

Neutral lipids (mainly triacylglycerols) are generally highest in the flesh and least in the liver. Correspondingly, the levels of polar lipids (mainly PC and PE) are lowest in the flesh and highest in the liver.

(ii) Component fatty acids

Variations occur in the fatty acid profiles related to lipid class and site.

The proportion of saturated acids and of the major component palmitic acid, is highest in the phosphatidyl cholines at all three sites and lowest in the phosphatidyl ethanolamines.

In the flesh and liver lipids, monoene acids are highest in the triacylglycerols and much lower in the phosphatidyl cholines and phosphatidyl ethanolamines. These differences are retained in the gonad lipids, but are less marked.

At all three sites the n-6 polyene acids attain their highest levels in the triacylglycerols and cardiolipins and their lowest levels in the phosphatidyl cholines. The levels in the phosphatidyl ethanolamines are much closer to the phosphatidyl cholines in the flesh, and to the triacylglycerols in the liver and gonads.

This situation is largely reversed with the n-3 polyene acids. Very high levels of these acids are present in both groups of phosphatidyl esters. Values are lower for the triacylglycerols, but

the differences are less marked in the gonad lipids than in the lipids from flesh and liver.

The detailed figures in Table 10 are reduced to their simplest form, in a modification which makes these two-fold comparisons more comprehensible.

(iii) Farmed and wild fish

The gonads have a lower total lipid content for farmed compared to wild salmon, whereas the flesh and liver are similar. This difference is reflected particularly in the TG level of wild salmon gonads, which is greatly increased. The relative proportions of the other components are decreased (although their absolute amounts are increased).

In terms of fatty acid composition, the most striking difference is the higher level of 18:2 n-6 in the TG (and PE,CL) of flesh, liver and gonads of farmed salmon compared to wild. Less striking differences occur for n-3 polyene acids of CL (which are less for farmed fish in flesh, liver and gonads) and for PE (greater for farmed fish in flesh, but less in gonads).

(iv) Effect of maturation

Immature farmed salmon which have spent eighteen months in sea water, under identical dietary and environmental conditions to those farmed for 12 months in sea water, have higher total lipid levels for their flesh, liver and gonad organs. This is reflected in an increased total neutral lipid level (mainly TG) over this period.

Table 10. Variation in fatty acid profiles in relation to lipid class and site

		TG	PC	PE	CL
16:0 + 18:0	F	14	16	11	15
	L	11	17	11	17
	G	12	22	13	19
16:1 + 18:1 + 20:1	F	38	9	13	27
	L	44	17	17	20
	G	27	20	17	26
18:2 + 20:4 (n-6)	F	14	4	5	13
	L	10	5	9	11
	G	9	4	8	3
20:5 + 22:5 + 22:6 (n-3)	F	22	67	61	30
	L	26	56	55	34
	G	45	49	53	32

These differences are perhaps not too surprising as the extra six months feeding time for the older farmed fish should produce a larger fish (assuming no sexual maturation) with concomitantly larger lipid stores (mainly neutral lipids).

Maturation causes a drop in the total lipid levels of the flesh, little change in the liver, but an increase in the gonads. These reciprocal changes in the lipid contents of flesh and gonads are probably a result of the general depletion of stored visceral lipid (including flesh lipids), transferred to the developing ovary (via the liver) during maturation. In each of the three lipid sites, the proportion of total neutral lipids is decreased and PC and (except for gonads) PE are increased upon maturation. The drop in neutral lipids in flesh and liver is mainly due to a decrease in the relative proportion of TG, but in the gonads TG is actually increased, although the fall in the remaining neutral lipids more than compensates for this.

The most marked changes which occur in the fatty acid composition of farmed salmon over the period 12 to 18 months (mature) in sea water, occur in the liver and the gonads. In general, the livers of mature fish have higher levels of C_{16} and C_{18} acids (saturated and monoenoic), but lower levels of C_{20} and C_{22} $\underline{n}$-3 polyene acids (particularly 22:6) in their polar lipid fractions compared to immature fish. This agrees with the findings of Akulin[69], who studied the changes in fatty acid composition of the polar lipids of liver and muscle of sockeye salmon during the spawning period. The author found selective mobilisation of these fatty acids above, in the liver, but not in the muscle.

In the gonads, the phosphatidyl cholines (not PE or CL) upon maturation show a decrease in the relative proportions of C_{16} and C_{18} acids (saturated and monoenoic) but an increase in the n-3 polyene acids. The hepatic and gonadal triacylglycerols upon maturation, show changes in the relative proportions of the above acids which are the exact opposite to those of PC (and PE, CL) in the liver and gonads (not PE, CL though).

(v) Influence of the diet

The fatty acid composition of the lipid reserves of many fish has been found[65] to partly reflect that of the dietary lipids eg. a high level of linoleic acid in the diet results in high levels of this acid in the depot lipids[70]. However, endogenous fatty acids also contribute to the depot lipids and it is not always possible to relate the depot lipid composition to that of the diet.

It does not seem unreasonable, however, to attribute most of the differences between the lipid compositions of (immature) farmed and wild salmon which have spent a similar period in sea water (12 months), to differences between the artificial and natural diets for these salmon. The diet of wild salmon in the sea has a high level of n-3 polyenes and a relatively low content of linoleic, reflecting the consumption of marine zooplankton[69] or smaller fish[65], their major foods. The diet of farmed salmon, as described earlier, is a mixture of vegetable oils, linseed oil and fish meal(s) and the major lipid constituent TG, has high levels of linoleic (and linolenic) acid derived from these ingredients. The triacylglycerols (depot lipids) of farmed salmon have far higher levels of linoleic acid

than those of the triacylglycerols of wild salmon, at all three lipid sites. This difference is almost certainly a reflection of the far higher dietary levels of this acid for farmed salmon compared to the levels it would receive under natural conditions. Comparison of Tables 2 (p.48) and 5(p. 59) reveals that the ratio of the sum of the major n-6 polyene acids (18:2 and 20:4) to the sum of the major n-3 polyene acids (20:5, 22:5 and 22:6) in dietary TG is similar to that of the flesh (lipid depot) TG of immature farmed salmon [12 months in sea water, A/A'j. However, although this ratio is similar for dietary PC, for farmed salmon PC (Table 7, p. 67) at all three lipid sites, this ratio is drastically reduced because of the greatly increased content of n-3 polyene acids. The same phenomena has been observed for the fatty acids of the lipids of cultured herring[71] fed a diet based on squid muscle, very different from the natural diet which consists mainly of calanoid copepods. The fatty acids of the neutral lipids tended to reflect the fatty acid spectrum of the diet, and were very different from the characteristic pattern of herring oils, whereas the phospholipids retained the fatty acid pattern of marine fish (high n-3 polyene acid content, particularly 20:5 and 22:6). The cultured herring had selected the available polyunsaturated fatty acids from the dietary pool and fat depots for the synthesis of phospholipids. As a result of this, the neutral lipids of liver and extra-hepatic tissues had lower levels of 20:5 and 22:6 n-3 than either the diet or the phospholipids. The liver neutral lipids had the highest oleic acid, but the lowest 20:5 and 22:6 n-3 acids.

This enrichment of the phospholipids with polyunsaturated acids at the expense of those of liver neutral lipids has also been observed for turbot[72] and plaice[11] and indeed was described earlier for the livers of farmed and wild salmon (see "Observations, (ii)].

(vi) Male vs female

No significant differences are observed in either total lipid levels or lipid composition between male and female farmed salmon (A' and A) in either the flesh or the liver. Marked differences are observed for male and female wild salmon, but as only single fish are being considered the results are hardly significant.

(vii) Individual differences

Three male and three female farmed salmon (A' and A respectively) which had spent the same period (12 months) in the same sea water pen on an identical diet, showed no great individual differences in total lipid levels or lipid composition in either the flesh or liver sites.

III. EXPERIMENTAL

1. General

(i) Solvents

Solvents were of reagent grade and redistilled before use.

Dry methanol was prepared in the following way.

Dry magnesium turnings (10 g) and methanol (50 ml) were warmed together with a few crystals of iodine. A vigorous reaction soon started and when all the magnesium had dissolved, further methanol (1.5 l) was added and the mixture refluxed for 1 hr, after which it was redistilled.

(ii) Chromatographic Analysis

(a) Thin Layer Chromatography

Analytical TLC was carried out on glass plates (20 x 5 cm) coated with silica gel G (0.25 mm wet thickness). For separations of lipids on a preparative scale, glass plates (20 x 20 cm) coated with a 0.5 mm thick layer of silica gel G or silica gel H (10% magnesium trisilicate) were used. After this preparation the TLC plates were dried at room temperature for 1 hr, activated at 110-120°C for 2 hr and stored in a drying cabinet containing silica. Prior to use, the TLC plates were reactivated for at least $\frac{1}{2}$ hr at 110-120°C.

Mixtures of petroleum (bp 40-60°), diethyl ether and glacial acetic acid or of chloroform, methanol and water (or aqueous ammonia) were normally used as developing solvents for TLC.

The components on analytical TLC plates were generally

detected by spraying with an ethanolic solution of phosphomolybdic acid (10%, w/v) and then heating at 110-120°C. Within a few minutes, separated lipids appeared as black spots on a greenish-yellow background.

More specific spray reagents were also used for identifying the major neutral and polar lipid components after TLC separation. When a solution of ferric chloride in water, acetic acid and sulphuric acid was used as spray reagent, cholesterol esters and free cholesterol (not other lipids) appeared as red-violet spots after heating at 100°C for 2-3 min.[73]

After spraying with the periodate-Schiff reagent, leaving for 15 min at room temperature and treating with sulphur dioxide to destroy excess reactant, lipids with a vicinal diol group such as phosphatidyl inositol appear as purple spots[74].

Phospholipids such as phosphatidyl ethanolamine and phosphatidyl serine, that have free amino groups, were detected with the aid of a ninhydrin spray, appearing as red-violet spots when the plate was heated in an oven at 100°C in a water-laden atmosphere[75].

Preparative TLC plates were sprayed with an ethanolic solution of 2',7'-dichlorofluorescein (0.2%, w/v) and viewed under ultraviolet light. The resolved components, which appeared as yellow bands on a purple background, were marked, scraped off the plate and extracted using different solvent systems for neutral and polar lipids. The solvent was removed under reduced pressure and the last traces were blown off with nitrogen.

(b) Argentation Chromatography

Ag^+ TLC was carried out on plates (20 x 20 cm) coated with silica gel G (0.5 mm thick) containing silver nitrate (10%). The plates were activated at 110°C for 2 hr after preparation, and if not used immediately they were stored in a desiccator protected from light. They were reactivated at 110°C for ½ hr before use.

Mixtures of petroleum (bp 40-60°) and diethyl ether were generally used as developing solvents. After development the plates were dried in a gentle stream of nitrogen and sprayed with an ethanolic solution of 2',7'-dichlorofluorescein (0.2%, w/v), and viewed under ultraviolet light. The separated components appeared as yellow bands on a purple background. They were marked, scraped off the plate and extracted with diethyl ether and/or chloroform-methanol (9:1, v/v).

(c) Gas Liquid Chromatography

GLC was carried out on a Pye series 104 chromatograph equipped with a flame ionisation detector. The glass columns (1.5 m long, 6 mm external diameter) contained the stationary phases 1) 10% SP-222PS, 2) 20% DEGS and 3) 20% EGSS-Y coated on Chromosorb W (100-120 mesh) supports. (The packing materials were obtained from Supelco, Inc.)

The oven temperatures employed for these three columns were 188°C, 185°C and 200°C respectively. Nitrogen, dried by passage through a column packed with molecular sieve type 4A, was used as the carrier gas at a flow rate of 40 ml/min for 1) and 60 ml/min for 2) and 3).

Quantitation of the peaks was based on peak height x retention distance and compositions of mixtures are reported as weight percentages. Straight chain saturated acids (16:0 to 24:0) were run as external standards and ECL values and percentage composition were calculated by a computer programme.

Tentative identification of the major polyene fatty acids present was made by comparing their ECL values with published reference tables for polyene acids[67] and by their relative retention ratios ie. retention distance of unknown peak/retention distance of reference peak.

(iii) Transesterification

The reagent used for transesterifying the separated lipid components from TLC was 5% (w/v) anhydrous hydrogen chloride in methanol, prepared by adding acetyl chloride (5 ml) slowly to cooled dry methanol (50 ml)[76]. This reagent was used in the following way.

The lipid sample (up to 50 mg) was dissolved in benzene (1 ml) in a test tube and 5% methanolic hydrogen chloride (2 ml) added. The mixture was refluxed for 2 hr then 5% (w/v) brine solution (5 ml) was added. The methyl esters were then extracted with diethyl ether (2 x 5 ml) using a Pasteur pipette. The ether layer was washed with water (4 ml) containing potassium bicarbonate (2%, w/v) and dried over anhydrous sodium sulphate . The excess solvent was removed under reduced pressure in a rotary film evaporator or in a stream of nitrogen.

No solvent (benzene) was necessary if phospholipids were transesterified.

2. Analytical

(i) Source of samples

Farmed and wild Atlantic salmon (Salmo salar) were sacrificed. Their flesh, liver and gonad organs were removed immediately and stored in chloroform-methanol (2:1, v/v) solution at -20°C, until they were required for analysis.

(a) Farmed Atlantic Salmon

Flesh (whole fillet), liver and gonad samples (female only) were obtained from farmed Atlantic salmon at Findon. These fish, both male and female, had spent approximately twelve months in sea water, but were still sexually immature. These Atlantic salmon (group A(female) and group A' (male)) lived in the same sea water pen and were fed exactly the same diet over the duration of their lives.

Another selection of flesh (tail fillet), liver and gonad samples were obtained from Atlantic salmon which had spent approximately eighteen months in sea water at Findon. These samples were all female, some were immature (group B) and some mature (group C). These fish had been fed a diet almost identical in composition to that given to the younger farmed salmon (12 months in sea water) from Findon.

(b) Wild Atlantic Salmon

One female (D) and one male (D') wild Atlantic salmon were captured out at sea off Aberdeen and flesh (whole fillet), liver and gonad samples were removed for analysis. These wild salmon had spent approximately twelve months in sea water, but were still

sexually immature.

Another (immature) wild Atlantic salmon (female (E)) which had spent approximately twenty-four months in sea water was also captured out at sea off Aberdeen and flesh (whole fillet), liver and gonads were removed for analysis.

(ii) Extraction of Lipids

(a) (Wet) Animal tissues

The fish tissues to be extracted had been previously stored in chloroform-methanol (2:1, v/v) solution at -20°C and were still "wet" from these solvents.

Two methods of extraction were used for isolating lipids from tissues, depending on the "wet" weight of tissue involved. For tissue weights $>$ 10 g the following procedure was used.

The tissue (100 g) was homogenised in a blender with chloroform (100 ml) and methanol (200 ml) for 2 minutes. The mixture was filtered through a sintered glass funnel and the tissue residue was rehomogenised in chloroform (100 ml) and filtered. The combined filtrates were transferred to a separating funnel, one quarter volume of potassium chloride solution (0.88%, w/v) added and the mixture shaken thoroughly. After separation and clarification ($\sim$15 min) the lower layer was removed, filtered, and the excess solvent removed in a rotary film evaporator under reduced pressure. The purified lipid was stored in a small volume of chloroform at -20°C, pending further analysis.

This procedure is based on the assumption that the tissue

contained 80% of water and it was important that the composition of the final biphasic chloroform-methanol-water solution was as close as possible to a 2:2:1.8 ratio (by volume). If necessary, more potassium chloride solution was added to attain this ratio. The original chloroform-methanol solution surrounding the stored tissue was included in the initial work-up procedure.

For tissue weights up to 10 g, the following extraction procedure was employed.

The tissue (1 g) was homogenised in a blender with methanol (10 ml) for 1 min, then chloroform (20 ml) was added and the process continued for a further 2 min. The mixture was filtered, the residue resuspended in chloroform-methanol (2:1 v/v, 30 ml) and rehomogenised for 2 min before filtration. The solid residue was washed with chloroform (20 ml) and methanol (10 ml), the combined filtrates transferred to a separating funnel and one-quarter volume of aqueous potassium chloride solution (0.88%, w/v) added. The contents of the funnel were shaken thoroughly, then the lower layer was removed and rewashed with one quarter volume of water-methanol (1:1, v/v). The purified lower layer was recovered and stored as described above.

All weights of extracted lipid have been recorded as g of lipid per g wet weight of tissue.

(b) "Dry" Diet sample

The total lipid was extracted from a sample of the diet fed to the farmed fish at Findon, by the following method.

The "dry" diet sample (50 g) was allowed to stand in chloroform (500 ml) for 30 min before blending for 2 min. The mixture was filtered through a sintered glass funnel and the residue washed with chloroform (500 ml). The filtrates were combined and the excess solvent removed. The extracted lipid was stored in the usual way.

(iii) Separation of Lipid Classes by Preparative TLC

(a) Neutral lipids

The extracted lipid (up to 50 mg) was separated into its component neutral lipids by chromatographing on silica gel G (0.5 mm) TLC plates in the solvent systems petroleum (bp 40-60^{o})-diethyl ether-acetic acid (80:20:1, v/v or 90:10:1, v/v). The mobilities of lipids in the former system were (in order of decreasing Rf value) hydrocarbons, cholesterol (wax) esters, triacylglycerols, free fatty acids, 1,3-diacylglycerols, cholesterol, 1,2-diacylglycerols and total polar lipids.

After development, the TLC plates were sprayed with 2',7'-dichlorofluorescein and examined under ultraviolet light. The bands were marked, scraped off and extracted using (1) diethyl ether and (2) chloroform-methanol (4:1, v/v) to ensure maximum recovery of neutral lipid. The amount of each neutral lipid, after recovery from the TLC plate, was determined by gravimetry.

Identification of the major neutral lipids present in salmon lipids (triacylglycerols, cholesterol and cholesterol esters) was

confirmed by comparison of R_f value using authentic standards in different solvent systems. For the sterols, further evidence of their identities was obtained by ferric chloride spray reagent, retention time (cholesterol) on GLC versus authentic cholesterol and for cholesterol esters, examination of their methyl ester derivatives on TLC. This revealed cholesterol and methyl ester spots.

(b) Polar lipids (phospholipids)

The total lipid extract (up to 50 mg) was separated into its component polar lipids by chromatography on silica gel H (0.5 mm) containing magnesium trisilicate (10%, w/v) using the developing solvent system chloroform-methanol-water (65:25:4, v/v). The relative mobilities of lipids in this system were (in order of decreasing R_f value) total neutral lipids, cardiolipin, phosphatidyl ethanolamine, phosphatidylcholine and a complex band which could have contained phosphatidyl inositol, phosphatidyl serine and sphingomyelin.

This last band was extracted and rechromatographed on silica gel H (0.5 mm) TLC plates in the solvent system chloroform-methanol-aqueous ammonia (28%, w/v) (65:40:5 v/v). The mobilities in this system were phosphatidyl inositol > phosphatidyl serine > sphingomyelin. All the phospholipids listed above were extracted from the TLC plates, after separation and spraying, using successively (1) chloroform-methanol-water (65:25:4, v/v) or chloroform-methanol-aqueous ammonia (65:40:5, v/v), (2) methanol, (3) methanol-water-acetic acid (94:5:1, v/v), and (4) chloroform-methanol-water (5:5:1, v/v).

An aliquot of each extracted lipid solution was removed for the estimation of polar lipid in terms of its phosphorus content, while the remainder was transesterified and examined on GLC.

Polar lipids were identified, both by their R_f values in different solvent systems against authentic standards and by specific spray reagents (see page 84)

Cardiolipin (diphosphatidyl glycerol), usually a minor constituent of the polar lipids, was identified also by determining its absolute content of phosphorus in comparison with standard PC and PE. Cardiolipin has approximately half the phosphorus content of these two per mole of lipid. Using accurately weighed samples of PC, PE and CL it proved possible to show that on the basis of their absolute P content per g lipid, cardiolipin's value was approximately half that of the other two.

(iv) Quantitation of Component Lipids

(a) Gravimetry (neutral lipids)

The component neutral lipid extracts from the TLC separation were firstly refiltered to remove last traces of silica gel or calcium sulphate (binder) then the majority of the excess solvent was removed under reduced pressure in a rotary film evaporator. When this volume was quite small the last few ml of the lipid solution were carefully transferred to an accurately weighed vial and the last traces of solvent removed by a stream of nitrogen. The vial was reweighed on the microbalance and the weight of neutral lipid was determined. Recoveries from TLC plates using this method were 95% or greater for neutral lipids.

(b) Phosphorus determination (phospholipids)

An aliquot of the extracted phospholipid solution from TLC was blown to dryness in a stream of nitrogen and digested to phosphate by heating firstly with sulphuric acid and then with perchloric acid. The phosphorus content was then determined by measuring the intensity of colour produced upon reaction with ammonium molybdate/ascorbic acid in acid solution.

Assuming an average phosphorus content of 4% in phospholipids, the total amount of a phospholipid present was determined on the basis of its phosphorus content after digestion. Precise experimental details are given below.

1) Reagents

All reagents were of Analar grade.

Ascorbic acid (10%) Ascorbic acid (10 g) was dissolved in distilled water (100 ml) and the solution was stored in a refrigerator.

Ammonium molybdate (2.5%)

Sulphuric acid (3M) prepared by diluting concentrated sulphuric acid (AR, 18 ml) by adding it slowly to water (90 ml) stirring constantly.

Reagent A (prepared freshly) prepared by mixing sulphuric acid (3M, 1 volume) with distilled water (2 volumes) and ammonium molybdate solution (2.5%, 1 volume). Ascorbic solution (10%, 1 volume) was added and the mixture was shaken well.

Perchloric acid 72% (AR)

Phosphorus standard (100 ppm) KH_2PO_4 (4.386 g) was dissolved in water and made up to 10 ml in a volumetric flask. 1 ml of this

solution was diluted to 1 l (100 ugP/ml). For use, 25 ml of this solution were diluted to 250 ml (10 ugP/ml)

2) Ashing the sample

The lipid sample (5-20 mg) or an aliquot from TLC) was placed into a borosilicate reaction tube and any excess solvent was blown off by a stream of nitrogen.

Concentrated sulphuric acid (4 drops) was added to each tube which were then placed in the already hot digestion apparatus (modified Kjeldahl rack). They were heated until white fumes of SO_3 appeared then perchloric acid (72%, 2 drops) was carefully added to each tube. If the contents of the tubes were not clear in 5 min, more perchloric acid was added and heating continued.

The tubes were removed from the heater after the digestion was completed and allowed to cool in the tube racks. When they were cool, distilled water was added and the contents transferred quantitatively to volumetric flasks (25 ml). These solutions were stable at room temperature for several days.

3) Measurement of phosphorus content

A quantity of the 25 ml digest solution coresponding to between 1 and 8 ug of phosphorus (or if the sample was completely unknown, 1 ml) was measured out.

These solutions were made up to 4 ml with distilled water. A blank containing 4 ml of water and standards containing 2 ug, 4 ug

and 6 ug of phosphorus (0.2, 0.4 and 0.5 ml of standard P solution) were also made up.

Freshly prepared reagent A (4 ml) was added to each tube, which were stoppered, mixed and kept at 37°C for $1\frac{1}{2}$ hr. The tubes were removed, cooled, and the absorbance/optical density was read in 1 cm silica cells at 820 nm against the blank. The reaction has a linear optical density vs P content relationship up to 8 ug of phosphorus and the phosphorus content could be determined from the standard curve.

Calculations

phospholipid (%) in total lipid

phosphorus (ug) in lipid (per mg) = $25\ A/VB$

phosphorus (%) in lipid = $2.5\ A/VB$

To convert to phospholipid (%) in total lipid, multiply by 25.

A = P(ug) from absorbance and standard curve

V = Volume of digest solution taken for measurement

B = Amount (mg) of lipid digested.

To calculate the absolute amount of phospholipid (mg) present in a digest ed aliquot (Cml) from the total phospholipid extract solution (Dml) from TLC.

Amount of P (ug) present in total TLC lipid extract = $25 \times \frac{A}{V} \times \frac{D}{C}$

Amount of P (mg) present in total lipid extract = $0.025\ \frac{AD}{VC}$

Amount of phospholipid present (mg) = $25 \times \frac{0.025\ AD}{VC}$

The reaction was sensitive down to about 0.5 ug. The reproducibility of the results obtained by this method was ± 5 % for duplicate phospholipid determinations.

(v) Argentation Chromatography of Methyl Esters

Several methyl ester samples (mainly of triacylglycerols) with a range of fatty acids typical of the majority of samples analysed, were applied to TLC plates coated with silica gel G (0.5 mm) containing silver nitrate (10%).

The developing solvent system petroleum (bp 40-60°)-diethyl ether (50:50, v/v) separated the total methyl ester samples into a number of bands whose composition was determined by GLC, after extraction from the TLC plates. Their relative mobilities (in order of decreasing R_f value) were saturated and monoenoic, dienoic, trienoic and polyenoic (4 to 6 double bonds) methyl esters. Saturated and monoenoic methyl esters were reseparated on Ag^+TLC by the solvent system petroleum-diethyl ether (90:10, v/v). Saturated and monoenoic methyl esters were extracted from the silica gel using diethyl ether only, whereas for dienoic, trienoic and polyenoic components diethyl ether and chloroform-methanol (9:1, v/v) were used.

GLC analysis of the extracted methyl esters from TLC, enabled tentative identified of the component methyl esters from each band. These findings are discussed in more detail in the section dealing with the "Identification of fatty acids" (page 37).

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